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FIELD AND LABORATORY INVESTIGATIONS TO EVALUATE THE INFLUENCE OF NITRILOTRIACETIC ACID (NTA) ON EUTROPHICATION

Pacific Northwest Water Laboratory
Corvallis, Oregon

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REPORT TO COMMISSIONER, FEDERAL WATER QUALITY ADMINISTRATION

By

NATIONAL EUTROPHICATION RESEARCH PROGRAM
PACIFIC NORTHWEST WATER LABORATORY, CORVALLIS, OREGON

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Introduction

The field and laboratory investigations described in this report were designed to evaluate, in a relatively short time, the potential impact of nitrilotriacetic acid (NTA) on lakes and on eutrophication in general. The experiments were specifically designed to determine the effect of NTA and its ultimate potential breakdown products, carbon and nitrogen, on algal growth and primary productivity as well as its effect on lake sediment-water interactions. Since NTA is considered a prime replacement for sodium tripolyphosphate in synthetic detergent formulations, the effect of phosphorus was included in the studies. Lakes and lake waters were selected for study on the basis of (1) trophic levels, ranging from ultra-oligotrophic to highly eutrophic; (2) differences in water quality and chemistry; and (3) location in different geographic areas.

According to these criteria, 10 lakes and one river located in California, Minnesota, North Carolina and Oregon were chosen. The field studies on the California lakes were under the direction of Professor Charles R. Goldman, University of California, Davis. The investigations on the North Carolina lakes were directed by Professor Charles M. Weiss, University of North Carolina, Chapel Hill. The laboratory assays on the California lake waters, the field and laboratory investigations on the Oregon lakes, and the sediment-water studies were carried out by the National Eutrophication Research Program at the Pacific Northwest Water Laboratory, FWQA. The investigations on the lake and river in Minnesota were conducted at the NERP Field Station at Ely, Minnesota. The results of the studies at the University of California, and the University of North Carolina are contained in the appendix to this report.

Laboratory and *In Situ* Assays

Laboratory and *in situ* algal assays (according to the Provisional Algal Assay Procedure[1]) were conducted on three Oregon lakes representing three trophic levels: oligotrophic (Waldo Lake); mesotrophic (Triangle Lake); and eutrophic (Cline's Pond). Nutrient concentrations in these lakes during the sampling period (June, 1970 - September, 1970) are shown in Table 1. Water samples were collected for laboratory assays concurrently with the conducting of *in situ* ^{14}C assays. Laboratory assays were also performed on waters from oligotrophic Lake Tahoe and eutrophic Clear Lake in California. In both the laboratory and *in situ* assays a series of water samples was inoculated (spiked) with the following: NTA, secondary sewage effluent resulting from settled primary effluent containing 5 mg NTA/l[2] (SE/NTA), tertiary sewage effluent from the latter (TE/NTA), secondary sewage effluent without NTA (SE), tertiary sewage effluent without NTA (TE), carbon, nitrogen and phosphorus. The concentration of 5 mg NTA/l was based on the estimate that 50 percent replacement of the sodium tripolyphosphate in detergent formulations

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1/ Joint Industry-Government Task Force on Eutrophication, February 1969

2/ Calculated as free acid, but added as trisodium monohydrate salt (7.27 g NTA Na_3. H_2O mg/l, 99 percent pure, is equivalent to 5.0 mg NTA/l).

would result in approximately this amount of NTA reaching sewage treatment plants if it were not degraded in the sewer system.

The treated sewage samples were obtained from two parallel bench-scale activated sludge systems being fed primary settled sewage; one influent contained 5 mg NTA/1 while the other did not. The tertiary effluents were obtained by alum flocculation of the secondary effluents. After a short period of acclimitization, the NTA was completely degraded in the activated sludge system.

Two types of laboratory assays were conducted: one in which *Selenastrum capricornutum*, a unicellular green alga, was used as the test organism and another using the indigenous algal species. The *in situ* assays were short-term, one to four hours, while the laboratory assays extended over a period of 21 days.

Results of Laboratory Assays

The effects of the various additions to Waldo Lake, Triangle Lake, Cline's Pond, Lake Tahoe and Clear Lake Waters on 21-day growth responses of *S. capricornutum* are shown in Figs. 1, 2, 3 and 4 respectively. The 21-day growth responses, as determined by fluorescence (Chlorophyll *a*), of the indigenous algae in Waldo Lake, Triangle Lake and Cline's Pond waters are shown in Table 2.

With the exception of the sewage effluents, the various additions to Waldo Lake water had no appreciable effect on either the growth of the test alga, *S. capricornutum*, or the indigenous algae. In Triangle Lake water and Cline's Pond water the secondary sewage effluents and phosphorus generally stimulated algal growth. In some samples, it appeared that NTA had either a slight stimulatory or slight inhibitory effect on algal growth. Growth stimulation by NTA, however, does not appear to be related to its degradation, i.e., the NTA does not appear to serve as a nitrogen or carbon source. There was no disappearance (degradation) in either Waldo Lake or Cline's Pond waters during the 21-day assay period. In Triangle Lake water a decrease in NTA concentration occurred over the 21 days, varying from a 20 percent decrease in August and September to 75 percent in June. However, growth stimulation in the presence of NTA was less in these waters than in Waldo Lake and Cline's Pond where no degradation was observed. Any algal growth stimulation or inhibition in the presence of NTA is probably due to the chelating properties of NTA and its interaction in the trace metal metabolism of the algae.

The effects of the various additions to Lake Tahoe and Clear Lake waters on the 21-day growth of the test alga, *S. capricornutum*, are shown in Fig. 4. In Lake Tahoe water, only the two secondary effluents (SE and SE/NTA) and phosphorus stimulated algal growth. In Clear Lake water NTA, secondary sewage effluent (SE), and tertiary sewage effluent (TE) resulted in some growth stimulation. NTA analysis during these experiments did not demonstrate degradation during the 21-day test period.

Results of *In Situ* Assays

The chemical, physical and biological data for each *in situ* experiment are shown in Tables 1-11 in the appendix. Carbon fixed (milligrams carbon per cubic meter per hour) for each of the tests in Waldo Lake, Triangle Lake, and Cline's Pond is shown in Figs. 5, 6, and 7 respectively. Numbers on the left of each addition to the test bottles indicate the amount of addition, as milligrams per liter in the case of the various chemicals and as percent volume in the case of the sewage effluent, made to each bottle. The symbol to the right of each set of additions is the value of the least significant difference at the five percent level. A (+) indicates a statistically significant increase in productivity, and a (-) indicates a statistically significant decrease.

In Waldo Lake the addition of NTA had no effect on carbon fixation in any of the four experiments. Generally, the only additions which stimulated primary productivity were the sewage effluents, phosphorus, and a combination of phosphorus and nitrogen. None of the additions inhibited primary productivity.

The mean productivity value in Triangle Lake (Fig. 6) in July, August and September was approximately 48 times greater than that of Waldo Lake for the corresponding months. The addition of 5.0 and 0.1 mg NTA/l in July and September, respectively, stimulated carbon fixation, but when 80 mg NTA/l was added (in error) in August there was a definite inhibition. While all of the additions stimulated carbon fixation in the July experiment, only the sewage effluents and a combination of nitrogen and phosphorus stimulated productivity in the August experiment. The same was true in September except that the addition of nitrogen alone also stimulated primary productivity.

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Cline's Pond was the most productive of the three lakes studied, being approximately 3000 times more productive than Waldo Lake (Fig. 7). It obviously has sufficient nutrients to support a heavy algal population. Only in the late July experiment did any additives significantly increase primary productivity. In the mid-July and August experiments some of the additions decreased productivity.

Experimental Basin Study

Two bottomless enclosures, 4.5 m x 4.5 m x 3 m deep, were installed in Cline's Pond. In one, a solution of NTA was sprayed on the surface to effect a final overall concentration of 5 mg NTA/1. The other enclosure was used as a control. The NTA was added on June 23, 1970 and oxygen and temperature profiles, from surface to bottom, were obtained weekly. Other chemical, physical and biological parameters were measured semi-monthly.

Results of Experimental Basin Study

The concentration of NTA at the surface and mid-depth decreased somewhat soon after initiation of the experiment (Fig. 8), due largely to mixing and diffusion. Little change occurred in the concentration of NTA in the water near the bottom until August. At the end of 60 days NTA was not detectable at any depth. Dissolved oxygen remained uniformly low in the bottom water (Fig. 9) and only once exceeded 2 mg/1. The rate of NTA disappearance was particularly rapid after the 45th day and from that point it was the same in both the upper well oxygenated and deeper poorly oxygenated zones.

Phytoplankton production was definitely greater in the enclosure containing NTA (Fig. 10). Also, compared to the control, total Kjeldahl nitrogen, organic nitrogen, volatile suspended solids and total carbon were all greater. As would be expected, water transparency was greater in the control enclosure (Fig. 11). The rate of primary productivity, as measured by ^{14}C fixation, was likewise much higher in the NTA treated enclosure. On August 10, at peak phytoplankton production, the maximum rate of carbon fixation was 280 mg $C/m^3/hr$ as compared with 30 mg $C/m^3/hr$ in the control enclosure.

The increased algal production in the NTA treated enclosure does not appear to be positively related to increased nutrient availability (nitrogen and carbon) resulting from NTA degradation. Comparison of Fig. 8 and Fig. 10 shows that the period of heaviest algal growth preceded the period of rapid NTA decrease.

Observations of the animal populations in the enclosures and in the pond itself did not indicate any detrimental effects by NTA on the indigenous fauna. Sizeable populations of a microcrustacean developed in both enclosures and in the pond. The NTA enclosure also contained large populations of tadpoles. Several rainbow trout trapped in the NTA enclosure at the time of installation died in late June when dissolved oxygen levels fell below 2 mg/l. At the same time, however, nearly all of the fish in the pond died also.

Shagawa Lake Studies

At Shagawa Lake, Minnesota, a series of *in situ* algal assays was carried out in 80-gallon plastic bags containing either Shagawa Lake water or Burntside River water. Additions, similar to those made in the other laboratory and *in situ* assays, were made to each of these waters and the bags incubated in Shagawa Lake. A total of five experiments, with durations of 13 to 32 days, was carried out. The numbers of basins, experimental water used, and additives employed in each experiment are summarized in Table 3.

Shagawa Lake is highly eutrophic and typically produces dense blue-green algal blooms. Burntside River, actually a channel connecting Shagawa and Burntside Lakes, contains oligotrophic water. During the period October 1968 - September 1969, total phosphorus in Shagawa Lake averaged 0.05 mg P/l, and in Burntside River, 0.01 mg P/l. Total nitrogen averaged 0.83 and 0.50 mg/l respectively.

Results

The algal growth response to additions of NTA was variable in both oligotrophic Burntside River water and eutrophic Shagawa Lake water. The results are summarized in Figs. 12, 13, 14 and 15 where production of chlorophyll *a* relative to various additives is presented as percent change, positive or negative, from the control (unaltered lake or river water). These values were derived from integration of curves of chlorophyll *a* variation with time and therefore represent net change over the entire experiment.

Algal growth response to NTA in Burntside River water was positive in some experiments and negative in others (Fig. 12). Some stimulation occurred in Experiments 1, 4 and 5, but inhibition was indicated in Experiments 2 and 3. In all experiments with Burntside River water, additions of NTA plus phosphorus resulted in a greater stimulation in algal growth than additions of NTA and phosphorus alone. Nitrogen and phosphorus added together, however, resulted in greater growth responses than combined additions of NTA and phosphorus.

Generally there was little difference in the positive growth responses to the addition of any of the sewage effluents (SE, SE/NTA, TE and TE/NTA) (Fig. 13). The high degree of response to tertiary effluents in Experiment 5 was due to improper functioning of the tertiary processing unit, which at that time was effecting only about 50 percent phosphorus removal.

Algal growth response to NTA in Shagawa Lake water (Fig. 14) was negligible in all instances, but as was the case with Burntside River water, NTA plus phosphorus produced a greater stimulation of growth then did either NTA alone or phosphorus alone. Also, as with Burntside River water, the addition of nitrogen plus phosphorus resulted in greater growth than additions of NTA plus phosphorus. In Experiments 4 and 5 the lake appeared to have been nitrogen limited and the addition of nitrogen resulted in a greater growth stimulation than either phosphorus or NTA. This is not uncommon in highly productive lakes which have become enriched by excessive phosphorus input; the oligotrophic water from Burntside River did not exhibit a similar response.

Unlike Burntside River water, the growth responses in Shagawa Lake water were always greater with secondary and tertiary sewage effluents derived from primary settled sewage containing NTA (Fig. 15). The interrelationships of the various additions to Burntside River water and Shagawa Lake water as they appeared in Experiment 3 are shown in Figs. 16 and 17 respectively.

The relationship of algal growth response to NTA suggested that NTA, after a lag period of variable length, was being degraded with the released nitrogen serving as a nutrient source. This interpretation is not supported, however, by analytical data on NTA levels in the experimental basins (Table 4). For example, in Experiment 3, the NTA levels remained high throughout the 20-day duration of the experiment but there was definite stimulation when NTA and phosphorus and NTA alone were added. On the other hand, in Experiment 4 the NTA levels fell to quite low values in the basins containing Shagawa Lake water as well as those containing Burntside River water, but there was no algal growth stimulation in Burntside River water by the addition of either NTA alone or NTA plus phosphorus. There was some growth stimulation when NTA plus phosphorus was added to the basin containing Shagawa Lake water. However, the growth peaked on day eight of the experiment and the NTA concentration in the basin was still high at that time. A considerably greater response was elicited by the addition of nitrogen alone than by the addition of NTA plus phosphorus.

Response to NTA plus phosphorus added to Burntside River water in Experiment 5 began after about one week and increased gradually for about an additional two weeks. There was little change after that. The addition of nitrogen plus phosphorus behaved similarly, but the growth response began on the first day. Since NTA disappearance in the basins containing Burntside River water was quite rapid (Table 4) it could be inferred that NTA was functioning as a nitrogen source. However, NTA also was reduced relatively quickly to nondetectable levels in the basins containing Shagawa Lake water, but there was essentially no stimulation in algal growth. On the other hand, algal growth was strongly stimulated by the addition of nitrogen alone and by nitrogen plus phosphorus. It is highly unlikely, therefore, that algal growth stimulation in the basin to which NTA plus phosphorus was added resulted from nitrogen being made available through NTA breakdown.

NTA-Sediment Interactions

The objectives of this brief study were to investigate (1) effects between sediments and NTA, such as the solubilization of metals by chelation with NTA and adsorption of NTA on sediments, and (2) rates of degradation or disappearance of NTA. Sediments were obtained from various sources where the metal content was believed to be higher than normal. These included sediments from lakes which had been treated with copper sulfate for algal control (Clear Lake, California; Monona, Big Cedar, and Pewaukee Lakes, Wisconsin); and those near industrial and mining outfalls (mercury in Detroit River; copper, lead, zinc and cadmium from Spring Bow River, Montana). The sediments from Wisconsin contained high concentrations of hydrogen sulfide and the Lake Monona sediment was highly calcareous.

Sediments with synthetic hard and soft lake water were used in the studies. The synthetic waters were made up using 20 mg Ca/l and 10 mg Mg/l for soft water, and 70 mg Ca/l plus 10 mg Mg/l for hard.

The solubility of calcium and magnesium in NTA solutions at pH 6-7 was checked. In solutions containing 8 mg NTA/l and up to 100 mg/l of calcium or magnesium, no precipitation occurred over a period of several weeks at room temperatures. NTA concentrations showed no change.

Degradation studies.

Degradation of NTA was investigated using combinations of synthetic lake water and sediments from the six sources. Ten mg NTA/l were added to each sediment-water combination. The mixtures were thoroughly stirred for five minutes and then permitted to stand quietly throughout the remainder of the experiment. During the first two days the NTA concentration decreased sharply in all experiments (except for Lake Monona), tended to level off for a time, and then decreased very rapidly to below detectable limits (Figs. 18 and 19). The rapidity with which the initial reduction in NTA occurred indicates the probability of its being the result of adsorption by particulate matter. This was tested by subsequent experiments in which NTA was added to mixtures of activated carbon and synthetic lake water and kaolin and synthetic lake water. NTA disappearance in the activated carbon mixture was extremely rapid (Fig. 18), but less so in the kaolin mixture. The levelling off period and subsequent final, complete disappearance of NTA were presumed to represent a period of microbial acclimatization followed by rapid biodegradation of the NTA.

The assumption of biodegradation during the second period of NTA decrease was investigated using ^{14}C-labelled NTA. The tagged material was added to Lake Pewaukee and Lake Monona sediment-synthetic lake water combinations. A container of sodium hydroxide was arranged over the surface so as to absorb any CO_2 released by breakdown of NTA. NTA concentrations were measured analytically throughout the experiments by a polarographic technique adapted to the specific problem. The results of duplicate experiments using Lake Monona sediments appear in Fig. 19. NTA concentrations changed little during the first seven days, and radioactive carbon uptake by the sodium hydroxide and particulate matter was correspondingly slight. After this first week, however, NTA concentrations decreased very rapidly, becoming undetectable in 10 days in one experiment and 13 days in the other. At the same time, ^{14}C uptake by the NaOH and particulate matter increased steadily. The close correlation between NTA disappearance and increased ^{14}C pickup by the NaOH strongly indicates that the decrease in NTA was due to actual biodegradation. The expected initial decrease in NTA such as was found in the experiments on the sediments which was attributed to adsorption, did not occur in any experiments involving Lake Monona. This seemingly anomalous behavior has not been explained.

Metal Solubilizing and Chelating Effects

In the experiments on solubilization of metals in various sediments mixed in synthetic hard or soft waters, the greatest solubilization in the presence of NTA (10 mg/1) occurred with the sediments from Silver Bow River, Montana and Detroit River, Michigan. Approximately the same amounts of copper, zinc and cadmium were solubilized from the sediment of Silver Bow River whether it was only stirred briefly at the onset of the experiment or stirred continuously (Table 5). In each case, the concentration of copper increased approximately 50 times over that in the control. The concentration of zinc was increased ten-fold, cadmium three-fold, and manganese slightly. Lead and iron were not solubilized in 20 hours, but longer stirring did increase the concentrations of these two metals in the water in both the presence of NTA and the control.

Results of studies on sediments for Detroit River, Big Cedar Lake, Lake Pewaukee, Clear Lake and Lake Monona appear in Table 6. NTA solubilized iron, manganese and copper from Detroit River sediments, but not mercury. This was surprising because NTA is known to complex with mercury and this sediment contained 5 mg Hg/kg of wet sediment. Also, the interstitial water contained 1 mg soluble Hg/1 before the NTA treatment.

TABLE 5

Solubilization of Metals in Silver Bow River Sediments by NTA, With and Without Prolonged Stirring

Metal	Stirred 5 Minutes Control Soft	 NTA Soft	 Control Hard	 NTA Hard	Stirred 20 Hours Control Soft	 NTA Soft	 Control Hard	 NTA Hard
Fe	70	50	45	45	20	30	20	40
Mn	490	720	540	600	980	980	1040	980
Cu	18	960	19	930	13	1100	13	1050
Cd	9	25	8	25	11	27	13	25
Zn	40	270	25	250	36	260	44	250
Pb	20	25	25	15	80	80	75	75

Control = No NTA

Soft = Synthetic soft water

Hard = Synthetic hard water

NTA Concentration = 10 mg/l

Metal Concentrations in Micrograms per liter

TABLE 6

Solubilization of Metals in Sediments by NTA

	Detroit River, Michigan				Big Cedar Lake, Wisconsin			
Metal	Control Soft	NTA Soft	Control Hard	NTA Hard	Control Soft	NTA Soft	Control Hard	NTA Hard
Fe	78	187	83	176	15	80	43	80
Mn	36	58	7	37	< 1	2	2	3
Cu	5	17	3	12	4	6	4	7
Hg	0.5	0.5	0.5	0.5				

	Lake Pewaukee, Wisconsin				Clear Lake, California			
Metal	Control Soft	NTA Soft	Control Hard	NTA Hard	Control Soft	NTA Soft	Control Hard	NTA Hard
Fe	<1	11	<1	9				
Mn	10	14	16	16				
Cu	8	9	6	11	4	8	3	6

	Lake Monona, Wisconsin			
Metal	Control Soft	NTA Soft	Control Hard	NTA Hard
Fe			1.3	1.0
Mn			0.1	0.2
Cu			0.1	0.1

Control = No NTA

Soft = Synthetic soft water

Hard = Synthetic hard water

NTA Concentration = 10 mg/l

Metal Concentrations in micrograms per per liter

Although a slight amount of copper (1-5 μg/l) was solubilized from sediments of Clear Lake and Big Cedar Lake, the concentration does not appear to be significant. The solubilization of iron, however, was significant in sediments from Big Cedar Lake and Detroit River, but not from Monona and Pewaukee Lake sediments. The latter two sediments contained high concentrations of sulfide which may have complexed the metals so that they were not readily chelated with NTA.

Summary

Field and laboratory investigations were conducted to evaluate the potential impact of nitrilotriacetic acid (NTA) on eutrophication. Studies included laboratory and in situ algal assays on 10 lakes and one river located in different geographic areas and of various degrees of water quality. A series of additions (spikes) were made to these waters, including NTA, nitrogen, phosphorus, carbon, secondary sewage effluent resulting from settled primary effluent containing NTA, tertiary treated effluent of the latter, secondary sewage effluent without NTA, and tertiary effluent without NTA.

Generally NTA had no dramatic effects on algal growth or primary productivity in the Minnesota, North Carolina or Oregon lakes. Where there were stimulatory or inhibitory effects, they appeared to be related to the chelating properties of NTA and interaction with algal trace metal metabolism. Stimulation of ^{14}C assimilation (primary productivity) was greatest in the California lake waters. These lakes generally tend to be nitrogen deficient and it could be implied that the NTA served as a nitrogen source for algal growth. This is highly unlikely, however, because during 21-day assays conducted at the Pacific Northwest Water Laboratory there was no disappearance of NTA in these same waters. Also, it has been demonstrated previously that algae cannot utilize NTA directly as a nitrogen source.

Thus the stimulation of ^{14}C fixation in these waters also is probably related to metal chelation effects.

Generally the stimulation of algal growth by phsophorus and by secondary sewage effluents was much greater than any stimulation resulting from NTA.

In the presence of lake and river sediments there appears to be an immediate, but slight, disappearance of NTA which is due to adsorption of the NTA on particulate matter. After acclimitization, there is a rapid disappearance of NTA and the results suggest this is due to biochemical (microbial) degradation.

Solubilization of metals in sediments varies from one location to another, but in these studies the metals from river sediments near industrial and mining outfalls seemed to be released more readily than those from the lake sediments. Copper, iron, manganese, cadmium and zinc were solubilized from sediments by NTA. Copper, however, in highly reduced conditions in lake sediments was not readily solubilized in the presence of NTA. Mercury was not solubilized from Detroit River sediments.

APPENDIX A

Tables 1-11: The chemical, physical and biological data for each *in situ* experiment in Waldo Lake, Triangle Lake and Cline's Pond.

TABLE 1

Range of Chemical Concentrations (milligrams per liter)
in Test Lakes During Sampling Period
(6/70-9/70)

Chemical	Waldo Lake	Triangle Lake	Cline's Pond
Total Inorganic Carbon	<1.0	5.0-6.0	11.0-14.0
Total Organic Carbon	<1.0	1.0-2.0	7.0-9.0
NH_3-Nitrogen	<0.010	<0.001-0.030	0.01-0.07
NO_3-Nitrogen	<0.001-0.005	<0.001-0.010	<0.001-0.030
Kjeldahl-Nitrogen	<0.100	0.100-0.300	1.3-5.2
Ortho-Phosphorus	<0.001	<0.001-0.008	<0.01-0.27
Total-Phosphorus	<0.001	0.003-0.008	0.04-0.34
Total Iron	<0.050	<0.100-0.300	0.30-0.50

TABLE 2

Relative Growth Responses of Indigenous Algae to Various Additions in Natural Lake Waters

Lake	Date	Addition							
		NTA 5 mg/1	SE 2 %	SE/NTA 2 %	TE 2 %	TE/NTA 2 %	N .365 mg/1	C 1.9 mg/1	P 0.05 mg/1
Waldo	7/ 2/70	0	0	0	0	0	0	0	0
	7/27/70	0	++	0	0	0	0	0	0
	8/21/70	0	++	++	0	0	0	0	0
Triangle	6/23/70	-	0	0	0	-	0	0	+
	7/10/70	+	++	++	++	+	++		+
	8/ 7/70	0	++	+	0	0	0	0	0
	9/ 8/70	0	++	++	++	+	+	0	0
Cline's Pond	6/23/70	-	-	-	-	-	0	-	0
	7/17/70	0	+	+	+	+	0	0	0
	8/13/70	+	0	+	+	-	0	0	+

Legend: - = inhibition; 0 = no stimulation; + = slight stimulation; ++ = good stimulation.

TABLE 3

Summary of Experimental Designs, Experiments 1-5

Experiment No.	Dates of Experiment	Water Used	Additives* NTA	P	N	NTA + P	N + P	TERT WW	TERT WW NTA	SEC WW	SEC WW NTA	Control
1	June 3-17	Burntside	+	+	+	+	+			+		+
2	June 19 to July 7	Burntside	+	+	+	+	+			+		+
		Shagawa	+	+	+	+	+			+		+
3	July 14 to Aug. 3	Burntside	+	+	+	+	+	+	+	+	+	+
		Shagawa	+	+	+	+	+	+	+	+	+	+
4	Aug. 5 to Aug. 23	Burntside	+	+	+	+	+	+	+	+	+	+
		Shagawa	+	+	+	+	+	+	+	+	+	+
5	Aug. 27 to Sept. 28	Burntside	+	+	+	+	+	+	+	+	+	+
		Shagawa	+	+	+	+	+	+	+	+	+	+

*P added as K_2HPO_4

N added as KNO_3

NTA added as $NTA \cdot Na_3$

TABLE 4

NTA Analytical Values for Appropriate Basins in Experiments 3, 4, and 5

		BURNTSIDE RIVER				SHAGAWA LAKE			
	Basin No.	2	6	10	12	14	18	22	24
	Additive	NTA	NTA+P	TERT-NTA	SEC-NTA	NTA	NTA+P	TERT-NTA	SEC-NTA
Expt.3	July 14	5.6	4.4	<̄0.1	<̄0.1	4.6	5.8	<̄0.1	<̄0.1
	20	5.2	3.9	<̄0.1	<̄0.1	4.6	6.0	<̄0.1	<̄0.1
	24	4.4	<̄0.1	<̄0.1	<̄0.1	4.3	5.7	<̄0.1	<̄0.1
	30	3.8	3.4	<̄0.1	<̄0.1	4.3	6.0	<̄0.1	<̄0.1
	Aug 3	3.1	4.0	<0.4	0.8	4.1	5.2	<0.4	<0.4
Expt.4	Aug 5	5.8	3.7	<̄0.1	<̄0.1	3.5	5.9	<̄0.1	<̄0.1
	11	5.3	2.8	<̄0.1	<̄0.1	4.2	6.0	<̄0.1	<̄0.1
	17	4.7	0.2	<̄0.1	<̄0.1	3.7	5.5	<̄0.1	<̄0.1
	21	4.0	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1
	24	3.8	0.2	<̄0.1	<̄0.1	<̄0.1	0.5	<̄0.1	<̄0.1
Expt.5	Aug 26	4.0	4.5	—	—	5.3	5.1	—	—
	Sept 1	3.7	<̄0.1	0.9	0.9	4.4	3.6	—	—
	8	<̄0.1	<̄0.1	—	—	<̄0.1	5.0	—	—
	14	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<<̄0.1	—	—
	18	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1
	22	—	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1
	28	2.4	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1	<̄0.1

TABLE 1. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Waldo Lake - 6/23/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P
Chemical									
NH_3-N, mg/l	<.001		.006	.114	.011	.057			
NO_2-N, mg/l	<.001		.002	.009	.002	.0057			
NO_3-N, mg/l	<.001		.502	.479	.519	.519	.365		
Kjel -N, mg/l	.2		.219	.392	.202	.227			
total Phosphate (asP), mg/l	.002		.266	.247	.112	.116			
ortho Phosphate (asP), mg/l	.002		.241	.241	.112	.116			.051
total organic carbon, mg/l	3.		3.11	3.13	3.13	3.15			
total inorg. carbon, mg/l	.5		.58	.58	.56	.59		2.38	
pH	5.7								
total alkalinity, mg/l	2.5								
total hardness, mg/l	3								
total iron, mg/l									
NTA, mg/l		5		<.002		<.002			
Dissolved O_2, mg/l	10.9								
Physical									
Temperature, °C	6.5								
Submarine photometer:									
%of surface illumination;									
neutral filter	24								
green filter									
Secchi disc (20 cm) depth in meters	24								
Biological									
Chlorophyll a, mg/m^3	.12								
Bacteria: number/0.1 liter									
total plate count									
total coliform									
total fecal coliform									
Phytoplankton: predominant genera, number/ml									
Unidentified Green flagellates	44								
[illegible]	11								

TABLE 2. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Waldo Lake - 7/24/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P
Chemical									
NH_3-N, mg/l	<.01		<.03	<.03	<.03	<.03			
NO_2-N, mg/l	<.001		<.003	<.003	<.003	<.003			
NO_3-N, mg/l	<.001		.44	.44	.46	.46	.365		
Kjel -N, mg/l	<.1		<.1	<.1	<.1	<.1			
total Phosphate (asP), mg/l	<.01								
ortho Phosphate (asP), mg/l	<.003		.15	.14	.02	.01			.05
total organic carbon, mg/l	<1		<1	<1	<1	<1			
total inorg. carbon, mg/l	.5		.9	.9	.9	.9		2.38	
pH	5.8								
total alkalinity, mg/l									
total hardness, mg/l									
total iron, mg/l									
NTA, mg/l		5		<.002		<.002			
Dissolved O_2, mg/l	10.8								
Physical									
Temperature, °C	7.6								
Submarine photometer: %of surface illumination;									
neutral filter	27								
green filter	24								
Secchi disc (20 cm) depth in meters	29								
Biological									
Chlorphyll a, mg/m^3	.04								
Bacteria: number/0.1liter									
total plate count									
total coliform									
total fecal coliform									
Phytoplankton: predominant genera, number/ml									
Unidentified Green Flagellate	55								
Melosira	11								

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TABLE 3. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Waldo Lake - 8/19/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P	P+N
Chemical										
NH_3-N, mg/l	<.01		<.01	<.03	<.01	<.01				
NO_2-N, mg/l	<.001		<.0012	<.001	<.003	<.001				
NO_3-N, mg/l			.46	.54	.99	.53	<.04			<.04
Kjel -N, mg/l	.4		.42	.42	.41	.41				
total Phosphate (asP),mg/l										
ortho Phosphate (asP),mg/l	.006		.156	.166	.007	.007			.056	.056
total organic carbon,mg/l	2.		2.08	2.08	2.05	2.04				
total inorg. carbon, mg/l	.5		.7	.8	.9	.9		.54		
pH	5.7									
total alkalinity, mg/l	5.7									
total hardness, mg/l	1.									
total iron, mg/l	<.04									
NTA, mg/l		.01		<.002		<.002				
Dissolved O_2, mg/l	10.6									
Physical										
Temperature, °C	8.8									
Submarine photometer: %of surface illumination;										
neutral filter	22									
green filter	22									
Secchi disc (20 cm) depth in meters	29									
Biological										
Chlorophyll a, mg/m³	.07									
Bacteria: number/0.1 liter										
total plate count	400									
total coliform	<2									
total fecal coliform	<2									
Phytoplankton: predominant genera, number/ml										
-Unidentified green coccoid	11									
Unidentified green flagellate	11									

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TABLE 4. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Waldo Lake - 9/22/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P	P+N
Chemical										
NH_3-N, mg/1	.01		.01	<.03	.01	.01				
NO_2-N, mg/1	<.001		<.0012	<.001	<.003	<.0013				
NO_3-N, mg/1	<.001		.46	.54	.99	.53	.007			.007
Kjel -N, mg/1										
total Phosphate (asP), mg/l	.003		.15	.16	<.002	.002				
ortho Phosphate (asP), mg/l	<.002		.15	.16	.002	.002			.05	.05
total organic carbon, mg/1	<1									
total inorg. carbon, mg/1	.5		.7	.8	.9	.9		.54		
pH	6.0									
total alkalinity, mg/1										
total hardness, mg/1										
total iron, mg/1										
NTA, mg/1		0.1		<.002		<.002				
Dissolved O_2, mg/1	9.3									
Physical										
Temperature, °C	9.4									
Submarine photometer:										
% of surface illumination:										
neutral filter	21.5									
green filter	23									
Secchi disc (20 cm) depth in meters	22									
Biological										
Chlorophyll a, mg/m³	.35									
Bacteria: number/0.1 liter										
total plate count	<100									
total coliform	<1									
total fecal coliform	<1									
Phytoplankton: predominant genera, number/ml										
Unidentified green flagellate	50									
Melosira	11									

TABLE 5. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various In Situ Bottles Triangle Lake - 7/07/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P
Chemical									
NH_3-N, mg/l	.01		.04	.01	.013	.016			
NO_2-N, mg/l	.001		.003	.004	.003	.008			
NO_3-N, mg/l	.001		.532	.001	.001	.001	.366		
Kjel -N, mg/l	.3								
total Phosphate (asP), mg/l	.017								
ortho Phosphate (asP), mg/l	.007		.155	.155	.072	.070			.057
total organic carbon, mg/l	<1		<1	<1	<1	<1			
total inorg. carbon, mg/l	5		5.5	5.6	5.5	5.6		6.88	
pH	7.3								
total alkalinity, mg/l	17								
total hardness, mg/l									
total iron, mg/l									
NTA, mg/l		5		<.002		<.002			
Dissolved O_2, mg/l									
Physical									
Temperature, °C	19								
Submarine photometer:									
%of surface illumination:									
neutral filter	11.2								
green filter									
Secchi disc (20 cm) depth in meters	2.2								
Biological									
Chlorophyll a, mg/m^3	4.7								
Bacteria: number/0.1 liter									
total plate count	5600								
total coliform	98								
total fecal coliform	2								
Phytoplankton: predominant genera, number/ml									
Asterionella	330								
Unidentified green flagellate	264								
Chlamydomonas	66								

TABLE 6. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Triangle Lake - 8/05/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P	P+N
Chemical										
NH_3-N, mg/l	<.01		<.03	<.03	<.03	<.03				
NO_2-N, mg/l	<.001		<.003	<.003	<.003	<.003				
NO_3-N, mg/l	<.001		.44	.44	.46	.46	.365			.365
Kjel -N, mg/l	.4		.406	.406	.406	.402				
total Phosphate (asP), mg/l										
ortho Phosphate (asP), mg/l	<.001		.144	.137	.016	.011			.05	.05
total organic carbon, mg/l	<1		<1	<1	<1	<1				
total inorg. carbon, mg/l	4.4		4.8	4.8	4.8	4.8		6.3		
pH	7.4									
total alkalinity, mg/l	16									
total hardness, mg/l	14									
total iron, μg/l										
NTA, mg/l		80		<.002		<.002				
Dissolved O_2, mg/l	9.6									
Physical										
Temperature, °C	21.7									
Submarine photometer: % of surface illumination;										
neutral filter	22.4									
green filter	13.7									
Secchi disc (20 cm) depth in meters	3.5									
Biological										
Chlorophyll a, mg/m³	3.6									
Bacteria: number/0.1 liter										
total plate count	15400									
total coliform	26									
total fecal coliform	<2									
Phytoplankton: predominant genera, number/ml										
Anabena	220									
Unidentified green flagellate	154									
Cyclo-Steph	110									
Unidentified green coccoid	88									

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TABLE 7. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Triangle Lake - 9/02/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P	P+N
Chemical										
NH_3-N, mg/l	.01		.01	.03	.01	.01				
NO_2-N, mg/l	<.001		<.001	.001	<.003	<.001				
NO_3-N, mg/l	<.003		.46	.54	.99	.53	.01			.01
Kjel -N, mg/l	.4									
total Phosphate (asP), mg/l	.009									
ortho Phosphate (asP), mg/l	.003		.15	.16	.004	.004			.053	.053
total organic carbon, mg/l										
total inorg. carbon, mg/l	2.8		3	3.1	3.2	3.2		2.8		
pH	6.8									
total alkalinity, mg/l	15									
total hardness, mg/l	13									
total iron, mg/l	<.05									
NTA, mg/l		.01		<.002		<.002				
Dissolved O_2, mg/l	8.4									
Physical										
Temperature, °C	20.5									
Submarine photometer: % of surface illumination:										
neutral filter	11.0									
green filter	15.6									
Secchi disc (20 cm) depth in meters	2.5									
Biological										
Chlorphyll a, mg/m³	3.5									
Bacteria: number/0.1 liter										
total plate count	17500									
total coliform										
total fecal coliform										
Phytoplankton: predominant genera, number/ml										
Unidentified Green flagellate	330									
Unidentified green coccoid	176									
Chlamydomonas	66									

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TABLE 8. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Cline's Pond – 7/14/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P
Chemical									
NH_3-N, mg/l	.03		.03	.03	.03	.03			
NO_2-N, mg/l	<.01		<.01	<.01	<.01	<.02			
NO_3-N, mg/l	<.01		.53	<.01	<.01	<.01	.37		
Kjel -N, mg/l									
total Phosphate (asP), mg/l	.16								
ortho Phosphate (asP), mg/l	<.01		.15	.148	.07	<.06			.05
total organic carbon, mg/l	7		7.06	7.08	7.08	7.08			
total inorg. carbon, mg/l	18		18.5	18.6	18.5	18.6		19.9	
pH	10.9								
total alkalinity, mg/l	61								
total hardness, mg/l	39								
total iron, mg/l	0.5								
NTA, mg/l		5		<.002		<.002			
Dissolved O_2, mg/l	13.6								
Physical									
Temperature, °C	21								
Submarine photometer: %of surface illumination:									
neutral filter	9								
green filter									
Secchi disc (20 cm) depth in meters	.4								
Biological									
Chlorophyll a, mg/m³	21.1								
Bacteria: number/0.1 liter									
total plate count									
total coliform									
total fecal coliform									
Phytoplankton: predominant genera, number/ml									
Ankistrodesmus	240,200								
Scenedesmus	124,600								

TABLE 9. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Cline's Pond - 7/29/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P	P+N
Chemical										
NH_3-N, mg/l	.04		<.06	.06	<.06	<.06				
NO_2-N, mg/l	.001		<.003	.003	.003	<.003				
NO_3-N, mg/l	.006		.445	.445	.462	.466	.365			.365
Kjel -N, mg/l	1.4		1.46	1.41	1.41	1.40				
total Phosphate (asP), mg/l	.04									
ortho Phosphate (asP), mg/l	.007		.151	.144	.023	.018			.057	.057
total organic carbon, mg/l	5		5.04	5.06	5.04	5.04				
total inorg. carbon, mg/l	13		13.4	13.4	13.4	13.4		14.88		
pH	7.7									
total alkalinity, mg/l	46.8									
total hardness, mg/l	36									
total iron, mg/l	0.60									
NTA, mg/l		5		<.002		<.002				
Dissolved O_2, mg/l	7.6									
Physical										
Temperature, °C	22.0									
Submarine photometer: % of surface illumination;										
neutral filter	40.9									
green filter	38.5									
Secchi disc (20 cm) depth in meters	1.1									
Biological										
Chlorphyll a, mg/m^3	13.9									
Bacteria: number/0.1 liter										
total plate count	32800									
total coliform	110									
total fecal coliform	70									
Phytoplankton: predominant genera, number/ml										
Scenedesmus	4800									
Ankistrodesmus	2680									
Euglena	5120									

TABLE 10. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Cline's Pond - 8/11/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P	P+N
Chemical										
NH_3-N, mg/l	<.01		<.03	<.03	<.03	<.03				
NO_2-N, mg/l	.001		<.003	<.003	<.003	<.003				
NO_3-N, mg/l	.002		.441	.441	.458	.462	.367			.367
Kjel -N, mg/l	1.1		1.15	1.11	1.11	1.1				
total Phosphate (asP), mg/l	.006									
ortho Phosphate (asP), mg/l	.009		.156	.146	.025	.020			.059	.059
total organic carbon, mg/l	7		7.04	7.06	7.04	7.04				
total inorg. carbon, mg/l	11		11.4	11.4	11.4	11.4		12.88		
pH	8.8									
total alkalinity, mg/l	41.3									
total hardness, mg/l										
total iron, mg/l										
NTA, mg/l		5		<.002		<.002				
Dissolved O_2, mg/l	8.6									
Physical										
Temperature, °C	23.0									
Submarine photometer:										
%of surface illumination:										
neutral filter	26.7									
green filter										
Secchi disc (20 cm) depth in meters	1.4									
Biological										
Chlorphyll a, mg/m³	8.3									
Bacteria: number/0.1 liter										
total plate count	870000									
total coliform	58									
total fecal coliform	38									
Phytoplankton: predominant genera, number/ml										
Scenedesmus	3320									
Euglena	2560									
Ankistro[illegible]mus	800									

TABLE 11. Environmental Parameters of Control Water and Final Concentration of Selected Chemical Substances in the Various *In Situ* Bottles Cline's Pond - 9/10/70

PARAMETER	CONTROL	NTA	S.E.	SE/NTA	T.E.	TE/NTA	N(NTA)	C (NTA)	P	P+N
Chemical										
NH_3-N, mg/l	<.01		<.03	<.03	<.03	<.03				
NO_2-N, mg/l	.001		.001	.001	<.003	.001				
NO_3-N, mg/l	.002		.462	.545	.99	.534				
Kjel -N, mg/l	1.1		1.102	1.12	1.106	1.106	.009			.009
total Phosphate (asP), mg/l										
ortho Phosphate (asP), mg/l	.273		.423	.433	.274	.273				
total organic carbon, mg/l	5.		5.27	5.28	5.26	5.24			.32	.32
total inorg. carbon, mg/l	14		14.0	14.1	14.2	14.2				
pH	7.8							13.84		
total alkalinity, mg/l	56									
total hardness, mg/l	43									
total iron, mg/l	3.3									
NTA, mg/l						<.002				
Dissolved O_2, mg/l	2.8	.01		<.002		<.002				
Physical										
Temperature, °C	19.5									
Submarine photometer: %of surface illumination:										
neutral filter	20.8									
green filter	15.7									
Secchi disc (20 cm) depth in meters	1.2									
Biological										
Chlorophyll a, mg/m³	13.2									
Bacteria: number/0.1 liter										
total plate count	25000									
total coliform	5									
total fecal coliform	<5									
Phytoplankton: predominant genera, number/ml										
Chlamydomonas	3740									

APPENDIX B

University of California, Davis Report

"The Response of Naturally Occurring Phytoplankton Populations to NTA in Lake Tahoe, Castle Lake, and Clear Lake, California."

Appendix B

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THOMAS E. MALONEY

The Response of Naturally Occurring Phytoplankton Populations to NTA in Lake Tahoe, Castle Lake, and Clear Lake, California

ABSTRACT

In situ and laboratory experiments designed to measure the effects of the addition of NTA and NTA-containing sewage effluents upon natural freshwater phytoplankton populations were performed. Data is presented from cultures of oligotrophic Lake Tahoe, mesotrophic Castle Lake and highly eutrophic Clear Lake. Significant stimulation of photosynthesis, as measured by C^{14} assimilation, was observed in these experiments with additions of NTA at low concentrations. The response to NTA additions was greatest in waters of low inorganic nitrogen content. In addition to perhaps supplementing the direct nitrogen requirements of the lakes' natural phytoplankton populations, the chelation properties of NTA may increase the availability of other necessary and perhaps limiting trace elements. Further, metals like copper which may be at near toxic levels in Clear Lake, might be rendered less toxic by the presence of NTA.

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The Response of Naturally Occurring Phytoplankton Populations to NTA in Lake Tahoe, Castle Lake, and Clear Lake, California

by

Charles R. Goldman and Dennis Fujita
Limnological Research Group, Institute of Ecology
University of California, Davis

INTRODUCTION

The mounting concern about the eutrophication of surface waters has directed attention toward the high phosphate content of conventional detergents since phosphorus has long been considered a major limiting factor in aquatic environments. High phosphate concentrations are often found in very eutrophic waters. Limiting factor work at the University of California, Davis has identified a number of different limiting factors in Alaska, New Zealand and California lakes. Molybdenum, for example, was found to be a limiting factor in Castle Lake, California in combination with potassium and sulfer (Goldman, 1960). Clear Lake, the largest lake entirely within the boundaries of California, is characterized by extremely high phosphate levels with nitrogen the most limiting of the spectrum of stimulatory chemicals (Goldman and Wetzel, 1963). Lake Tahoe, long renowned for its remarkable clarity and low nutrient content, is primarily limited by iron (Goldman, 1963) and nitrogen (Goldman and Armstrong, 1969).

Because of the extensive limnological research that has been done with these three lakes, they were selected for bioassay experiments with NTA which has been proposed as a phosphate substitute in the detergent industry. During the summer and fall of 1970 culture experiments with natural lake populations were conducted to determine the influence of NTA on algal growth. NTA in various forms for these experiments was supplied by the Corvallis laboratory of the FWQA. Natural populations of phytoplankton were used exclusively in these experiments without concentration. Both laboratory and in situ culturing was employed.

METHODS

The carbon-14 bioassay techniques developed by Goldman (1960, 1963) were used in this study. Solutions of $Na_2C^{14}O_3$ of known activity were carefully premixed in the total culture medium prior to the addition of the NTA solutions being tested. The following amounts of C^{14} were added to each of the cultures:

1) Lake Tahoe	50 μc/liter (50 ml subsample filtered daily)
2) Castle Lake	8.0 μc/liter (50 ml subsample filtered daily)
3) Clear Lake	5.4 μc/liter (25 ml subsample filtered daily)

Carefully washed and autoclaved 500 ml Pyrex culture flasks were used in all experiments. Whether incubated in situ or in the laboratory, well-mixed subsamples of each culture were filtered through HA Millipore^R discs of 30 mm diameter daily. The experiments lasted from 4 to 7 days. The radioactivity of the organisms retained on these filters was measured with a thin window, automatic, gas-flow Geiger-Müller counter.

Nitrate determinations were made with the cadmium reduction method of Strickland and Parsons (1965). Phosphorus was determined (colorimetrically as the blue phosphomolybdate complex based on a method that closely paralleled that of Murphy and Riley (1962). Total iron was measured after conversion to the ferrous state with hydrochloric acid and hydroxylamine. The red iron (II) - bathophenauthroline complex extracted into isopentyl alcohol was measured colorimetrically at 533 nm. Dissolved inorganic carbon concentrations were determined by the infrared analysis method of Armstrong, Goldman and Fujita (1970).

All experiments used dilutions from 5.00 mg $Na_3NTA \cdot H_2O$/liter stock solutions (equivalent to 3.44 mg NTA/liter). Series #29500 - 29503 and #39500 - 39503 sewage effluents from the Corvallis laboratory pilot plant were used in these experiments. Chemical analyses of these effluents provided by the FWQA are given in Tables 1 and 2.

Exact details of the experimental procedures were sometimes modified for the different tests. Therefore, we are including a more detailed description of each experiment identified by number. Lake Tahoe studies included one in situ and one laboratory incubation experiment.

Lake Tahoe NTA Culture (LTC-IV-70)

July 27-31, 1970

Water was collected July 27 at a depth of 20 meters from the Index station. Chemical analyses of water collected at the same location July 24 for total phosphorus (3.8 ppb P), nitrate-nitrogen (2.1 ppb N), and inorganic carbon (916 mg C/l) were performed. After spiking with NTA and sewage effluent solutions, the samples were incubated in the laboratory at Lake Tahoe. Included in this study were 0.01% - 1.0% dilutions of the NTA stock solution and series #29500 - 29503 sewage effluents.

Lake Tahoe NTA Culture (LTC-V-70)

August 6-13, 1970

Water was collected at the Index station at a depth of 20 meters on August 6. The results of chemical analyses performed on water samples collected at the same place on three dates before and after August 6 are listed below:

	Aug. 3	Aug. 11	Aug. 15
Total Phosphorus (ppb P)	4.5	——	4.0
Nitrate (ppb N)	3.0	——	2.5
Total Iron (ppb Fe)	7.0	——	——
Inorganic Carbon (ppm C)	——	10.16	——

Culture flasks were incubated in situ for a seven day period and removed for daily filtrations of 50 ml aliquots. Dilutions of the NTA stock solution and series #29500 - 29503 sewage effluents from 0.01% to 10.0% were studied.

Castle Lake tests also included one in situ and one laboratory incubation experiment.

Castle Lake NTA Culture (CASL-2-70)

Sept. 27–Oct. 3, 1970

Water from a depth of 3 meters was collected at station VI on Sept. 27. The following water chemistry data was obtained:

Nitrate - N	less than 1 ppb
Soluble PO_4-P	less than 0.5 ppb
Total P	3.1 ppb
Total Fe	37 ppb
Inorganic C	3.4 ppm

Nineteen culture flasks were prepared containing 0.01% - 1.0% dilutions of the NTA stock solution and sewage effluents #29500 - 29503. Cultures were incubated in situ at Castle Lake for seven days and removed for daily subsampling of 50 ml aliquots.

Castle Lake NTA Culture (CASL-1-70)

Oct. 29-Nov. 4, 1970

On Oct. 25 water at a depth of 5 meters was collected from station VI.

Chemical analyses were performed for the following elements:

Soluble PO_4-P	0.5 ppb
Total Fe	16 ppb
Nitrate-N	less than 1 ppb
Inorganic C	4.1 ppm

Eight different solutions were prepared in triplicate. Additions of the NTA stock solution at the 0.2% - 7.0% levels and sewage effluents #39500 and #39502 at the 0.2% level were made. These samples were incubated in the Davis laboratory for seven days.

All three Clear Lake experiments were incubated at the laboratory at Davis under controlled light and temperature conditions for seven day periods.

Clear Lake NTA Culture (CLC-1-70)

Sept. 17-23, 1970

Water was collected Sept. 15 at the raft station using a sampler which isolated a continuous column of the top three meters of water. Water chemistry analyses and measurements of physical parameters yielded the following results:

Soluble PO_4-P	390 ppb
Total soluble P	404 ppb
Total P	431 ppb
Total Fe	2.40 ppm
Inorganic C	25.3 ppm
Nitrate - N	15 ppb
pH	8.75
Temperature	20.8° C
Secchi depth	0.33 meter
Depth of 1% light transmission	0.92 meter

Eleven different solutions were prepared in duplicate. These included 0.1%, 1.0%, 5.0% and 10.0% dilutions of the stock NTA solution and 0.2% dilutions of the #29500 - 29503 sewage effluents.

Clear Lake NTA Culture (CLC-2-70)

Oct. 13-19, 1970

Water was collected Oct. 3 at Corinthian Bay and stored nine days before use. No chemical analyses were performed. Culture flasks were incubated for two days at ~22°C in a fume hood under fluorescent lighting. They were then transferred to an incubator at 17°C under improved lighting conditions for the remaining five days. Sewage effluents from the #39500 - 39503 series were used.

Clear Lake NTA Culture (CLC-3-70)

Oct. 29-Nov. 4, 1970

Water was collected at a depth of 0.5 m from the raft station on Oct. 27. Sample collection followed by one week the first rainfall of the season. The autumn bloom of *Aphanizomenon* which had initiated approximately one month earlier was still in evidence. A marked increase in nitrate concentrations over that of CLC-1-70 was observed.

Soluble PO_4-P	303 ppb
Total P	316 ppb
Total Fe	2.62 ppm
Nitrate - N	300 ppb
Temperature	16.3°C

RESULTS AND DISCUSSION

The bioassays of Lake Tahoe phytoplankton showed that NTA and the sewage effluents containing it generally stimulated C^{14} uptake (Table 3, Fig. 1-8). In the first experiment, LTC-IV-70, all additions of secondary and tertiary effluents both with and without NTA were stimulatory. At the end of the four day study, the cultures with effluents containing NTA at the $2 \times 10^{-3}\%$ and $2 \times 10^{-4}\%$ levels showed more assimilated C^{14} than did those containing the same concentration of effluents without NTA. At the highest concentration of $2 \times 10^{-2}\%$ the reverse situation was observed with this level producing definite inhibition. Additions of the NTA stock solution were stimulatory at the 0.1% and 1.0% levels while the 0.01% (0.344 ppb NTA) showed no significant change from control values.

The second Lake Tahoe experiment, LTC-V-70, also included additions of NTA and sewage effluents at the $2 \times 10^{-1}\%$ level. Incubation for this test was carried out in situ over a seven day period. Additions of tertiary effluents with and without NTA were all stimulatory to carbon-14 uptake in the concentration range tested ($2 \times 10^{-4}\%$ to $2 \times 10^{-1}\%$). All secondary effluent additions with NTA were also stimulatory; lack of stimulation was exhibited only by the two most dilute solutions of secondary effluent which did not contain NTA. Additions of the NTA stock solution at 0.01%, 0.1%, 1.0%, and 10.0% levels also increased C^{14} uptake with greatest photosynthetic enhancement shown at the 1.0% level of addition (34.4 ppb NTA).

Of all our experiments the in situ Castle Lake culture (CASL-2-70) produced the most significant stimulatory effects with NTA addition (Figs. 9-11). In Table 4 are listed the approximate levels of maximum stimulation (+) or inhibition (-) observed for samples when compared to the controls. All tertiary effluent additions gave positive responses, but those which contained NTA were the most stimulating. For the samples with secondary affluents added, only the ones with NTA were stimulatory. The cultures responded with increased C^{14} uptake only to the addition of the stock NTA solution at the 0.01% level. In the range tested the higher concentrations were not found to be stimulatory.

The second Castle Lake experiment, CASL-1-70, was incubated in a laboratory at Davis. All of the eight different cultures spiked with NTA or secondary sewage effluents (#39500 and 39502) responded with increased C^{14} uptake over the control after seven days of incubation (Table 4, Figs. 12, 13, 14). The secondary effluents with and without NTA were more effective at enhancing C^{14} assimilation than NTA alone.

All three Clear Lake NTA culture experiments were performed under light- and temperature-controlled conditions at Davis. Effects from the addition of NTA and sewage effluents were less noticeable and more variable in nutrient-rich Clear Lake water compared to Lake Tahoe or Castle Lake. Variations in the results from these three experiments may be explained on the basis of significant changes in phytoplankton and chemical composition of Clear Lake water during the period tested. Further the filimentous bluegreen algae (*Aphanizomenon*) clumps and therefore provides more sampling error than the more dilute Castle and Tahoe populations.

Greatest positive responses to NTA were observed for the first Clear Lake test, CLC-1-70 (Table 5, Figs. 15 and 16). Additions of the 5.00 mg $Na_3NTA \cdot H_2O$/liter stock solution at 0.1%, 1.0%, 5.0% and 10.0% levels stimulated C^{14} assimilation as did the two sewage effluents containing NTA. The amount of stimulation (+) or inhibition (-) shown by each culture is listed in Table 5. The highest stimulation was exhibited by the medium containing the NTA solution at the 1.0% level. At the end of the seven-day incubation period, the NTA-free sewage effluents (#29500 and 29502) both showed slight inhibition at the 0.2% level although definite stimulation had been observed for the first six days (Fig. 16).

Because of the possibility of non-photosynthetic adsorption of ^{14}Carbon in experiments of this type (Goldman and Mason 1962), both the 0.45 micron Millipore^{R}-filtered lake water plus 1.0% NTA solution and the unfiltered autoclaved lake water plus 1.0% NTA solution were checked for C^{14} uptake during the seven-day period. Carbon-14 uptake by these adsorption controls was found to be negligible.

At the completion of the second Clear Lake test, CLC-2-70, all samples showed stimulation of C^{14} uptake over the controls (Fig. 17, 18). Highest positive responses were found for the NTA stock solution at the 0.2% level (+33%), 0.2% secondary effluent (+18%) and 0.2% secondary effluent plus NTA (+14%). The culture flasks containing additions of the NTA stock solution showed initial inhibition at the 4.0% and 10.0% levels. After what appeared to be acclimatization periods of five and seven days, respectively, the organisms in these cultures were able to assimilate C^{14} as well as the controls. In contrast, the more dilute NTA solutions showed immediate accelerated rates to C^{14} assimilation.

The third Clear Lake test, CLC-3-70 was unique in that neither inhibition nor stimulation could be attributed to the added NTA or sewage effluents. Histograms of the seven day experiment show no really definite trends when compared to the control (Figs. 19, 20, 21, 22, 23). The variations observed are within the limits of experimental error. The absence of a measurable effect suggests that an entirely adequate supply of nutrients was present in the natural lake water. Except for a twenty-fold increase in the nitrate-nitrogen concentration, all chemical parameters measured were very similar to those for CLC-1-70. This definitely suggests the involvement of nitrogen in the earlier stimulation recorded and follows the upward shift in the nitrogen to phosphorus ratio which preceded this experiment.

CONCLUSIONS

The experimental results reported here demonstrate the stimulatory effect of effluents containing NTA on the photosynthetic activity of natural phytoplankton populations. This is not surprising when one conside that the three lakes assayed all tend to be nitrogen deficient, at least during the period when these experiments were conducted. Further, Lake Tahoe is a known iron deficient lake which may be affected by chelation. Chelation by NTA which may suppress inhibitory levels of copper in Clear Lake may be responsible in part for the high stimulation by NTA in Clear Lake phytoplankton.

ACKNOWLEDGEMENTS

The authors wish to acknowledge with thanks Bob Richards (Field Director at Lake Tahoe), Noel Williams (Field Leader at Castle Lake) and Hans Paerl, John Coil, Mike Shepard, and John Elder who provided laboratory and field assistance. This work was supported by FWQA Grant #16010 DBU. The Soap and Detergent Association kindly supplied the NTA and important field support for the Clear Lake studies.

TABLE I

CHEMICAL ANALYSES OF SERIES 29500 - 29503 SEWAGE EFFLUENTS

Substance	#29500	#29501	#29502	#29503
Total C (ppm)	21	24	22	?
Total organic C (ppm)	2	3	2	2
Total inorganic C (ppm)	19	21	20	?
Ammonia - N (ppm)	<1	<1	<1	<1
Nitrite - N (ppm)	<0.1	<0.1	<0.1	<0.1
Nitrate - N (ppm)	23.1	23.1	24	24.2
Kjeldahl - N (ppm)	3	0.3	0.3	0.1
Orthophosphate - P (ppm)	7.56	7.20	0.84	0.60
pH	7.3	7.3	7.0	7.3
Total Alkalinity (ppm)	59	64	82	82
Bicarbonate Alkalinity (ppm)	59	64	82	82
Dissolved Fe (ppb)	260	160	< 100	< 100
NTA (ppm)	<0.1	<0.1	<0.1	< 0.1

TABLE 2

CHEMICAL ANALYSES OF SERIES 39500 - 39503 SEWAGE EFFLUENTS

Substance	#39500 Secondary Effluent + NTA	39501 Tertiary Effluent + NTA	#39502 Secondary Effluent	#39503 Tertiary Effluent
Ammonia - N (ppb	60	60	60	120
Nitrite - N (ppb)	5	5	4	8
Nitrate - N (ppm)	42	40	44.1	42
Total P (ppm)	12	0.357	12	0.357
Orthophosphate - P	12	0.189	12	0.231
pH	7.1	7.4	7.3	7.3
Total Alkalinity (ppm)	64	54	75	75
Bicarbonate Alkalinity (ppm)	64	54	75	75
Total Fe (ppb)	600	80	360	80
NTA (ppm)	0.1	0.1	0.1	0.1

TABLE 3

EFFECTS OF NTA AND SEWAGE SPIKES ON LAKE TAHOE WATER

SUBSTRATE ADDED		% STIMULATION(+) OR INHIBITION (-) VS. CONTROL (LAST DAY OF EXPERIMENT)	
		LTC-IV-70	LTC-V-70
$2 \times 10^{-4}\%$	TERTIARY EFFLUENT	+59	+12
$2 \times 10^{-3}\%$		+9	+17
$2 \times 10^{-2}\%$		+117	+36
$2 \times 10^{-4}\%$	TERTIARY EFFLUENT + NTA	+100	+35
$2 \times 10^{-3}\%$		+13	+16
$2 \times 10^{-2}\%$		+99	+21
$2 \times 10^{-1}\%$		——	+61
$2 \times 10^{-4}\%$	SECONDARY EFFLUENT	+14	-9
$2 \times 10^{-3}\%$		+20	-35
$2 \times 10^{-2}\%$		+62	+4
$2 \times 10^{-4}\%$	SECONDARY EFFLUENT + NTA	+34	+24
$2 \times 10^{-3}\%$		+27	+48
$2 \times 10^{-2}\%$		+54	+57
$2 \times 10^{-1}\%$		——	+85
0.01%	NTA STOCK SOLUTION	-10	+21
0.1%		+37	+25
1.0%		+37	+58
10.0%		——	+48

TABLE 4

EFFECTS OF NTA AND SEWAGE SPIKES ON CASTLE LAKE WATER

SUBSTRATE ADDED		% STIMULATION (+) OR INHIBITION (-) VS. CONTROL (LAST DAY OF EXPERIMENT)	
		CASL-2-70	CASL-1-70
2×10^{-4}%	SECONDARY EFFLUENT	—	—
2×10^{-3}%		-2.5	—
2×10^{-2}%		-32	—
2×10^{-1}%		—	+175
2×10^{-4}%	SECONDARY EFFLUENT + NTA	+1430	—
2×10^{-3}%		+500	—
2×10^{-2}%		+920	—
2×10^{-1}%		—	+140
2×10^{-4}%	TERTIARY EFFLUENT	+550	—
2×10^{-3}%		+890	—
2×10^{-2}%		+1320	—
2×10^{-1}%		—	—
2×10^{-4}%	TERTIARY EFFLUENT +NTA	+1050	—
2×10^{-3}%		+740	—
2×10^{-2}%		+1430	—
2×10^{-1}%		—	—
0.01%	NTA STOCK SOLUTION	+1240	—
0.1%		-21	—
0.2%		—	+7
0.6%		—	+33
1.0%		+11	+35
3.0%		—	+20
7.0%		—	+43

TABLE 5

EFFECTS OF NTA AND SEWAGE SPIKES ON CLEAR LAKE WATER

SUBSTRATE ADDED	% STIMULATION (+) OR INHIBITION (-) VS. CONTROL (LAST DAY OF EXPERIMENT)	
	CLC-1-70	CLC-2-70
0.2% SECONDARY EFFLUENT	-2	+18
0.2% SECONDARY EFFLUENT + NTA	+40	+14
0.2% TERTIARY EFFLUENT	-3	+1
0.2% TERTIARY EFFLUENT + NTA	+41	+6
0.1% NTA STOCK SOLUTION	+22	—
0.2%	—	+33
1.0%	+50	—
2.0%	—	+9
4.0%	—	+5
5.0%	+21	—
10.0%	+34	0

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FIGURE 1

LAKE TAHOE NTA CULTURE (LTC-IV-70)

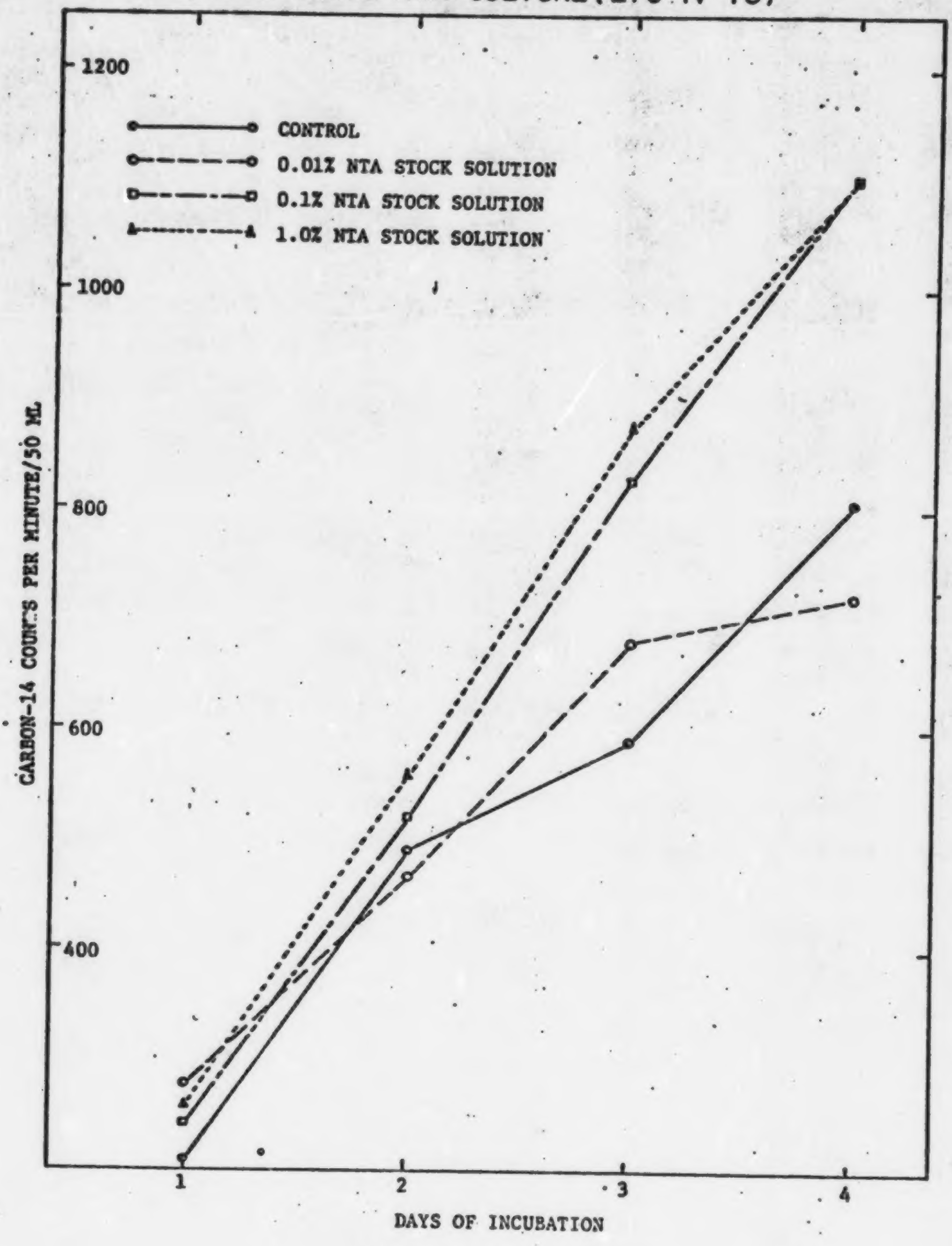


FIGURE 2

LAKE TAHOE NTA CULTURE(LTC-IV-70)

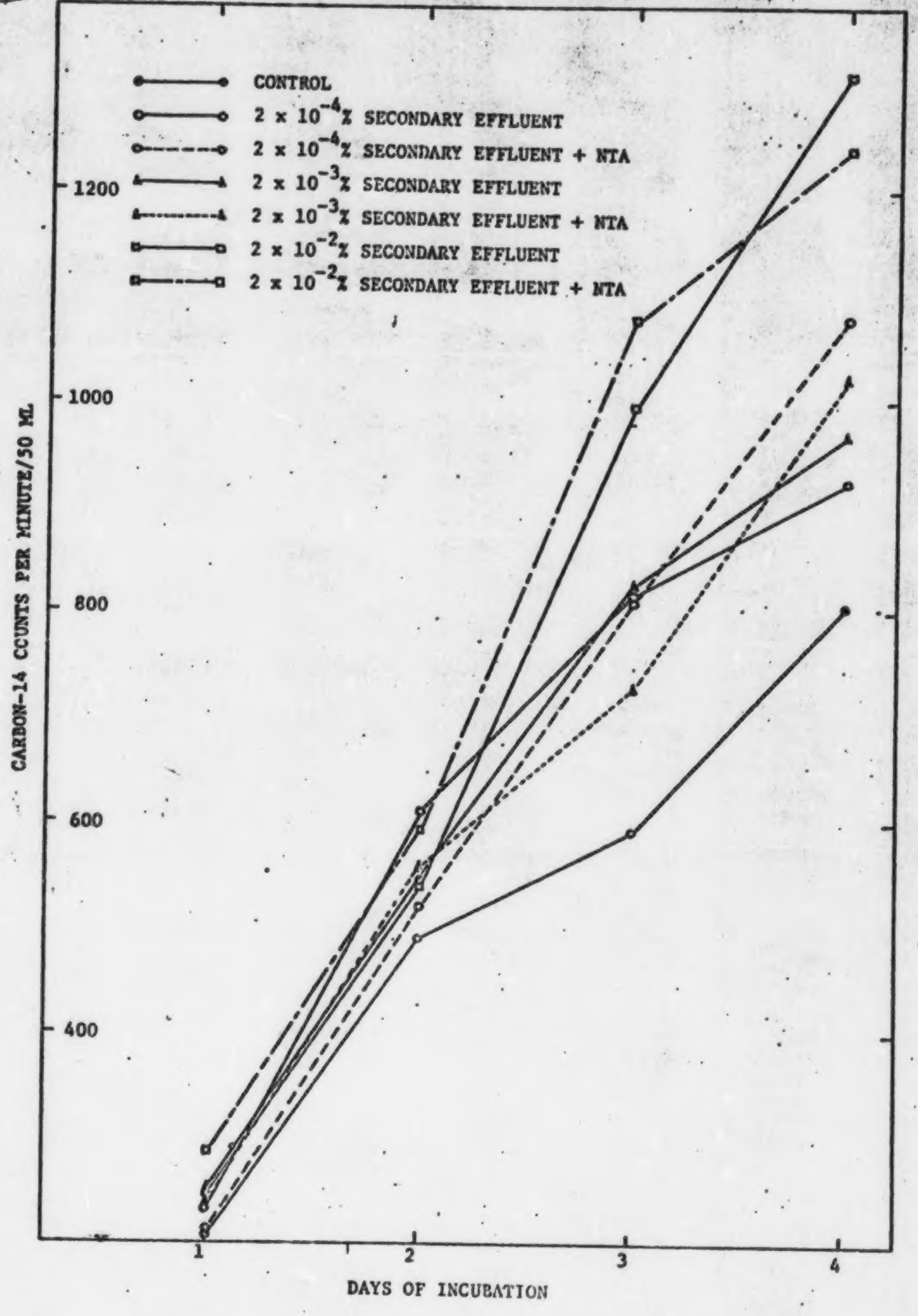


FIGURE 3

LAKE TAHOE NTA CULTURE (LTC-IV-70)

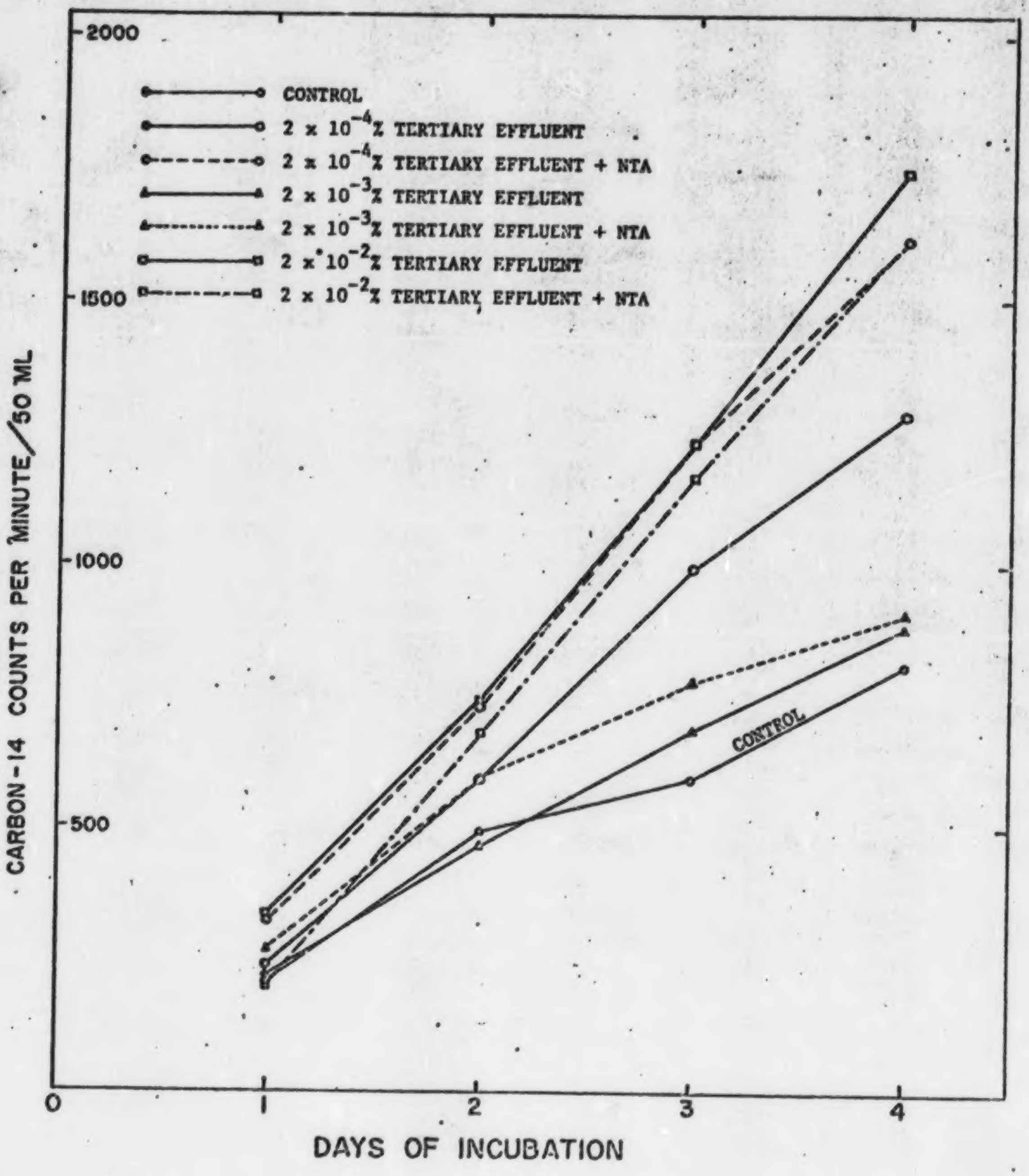
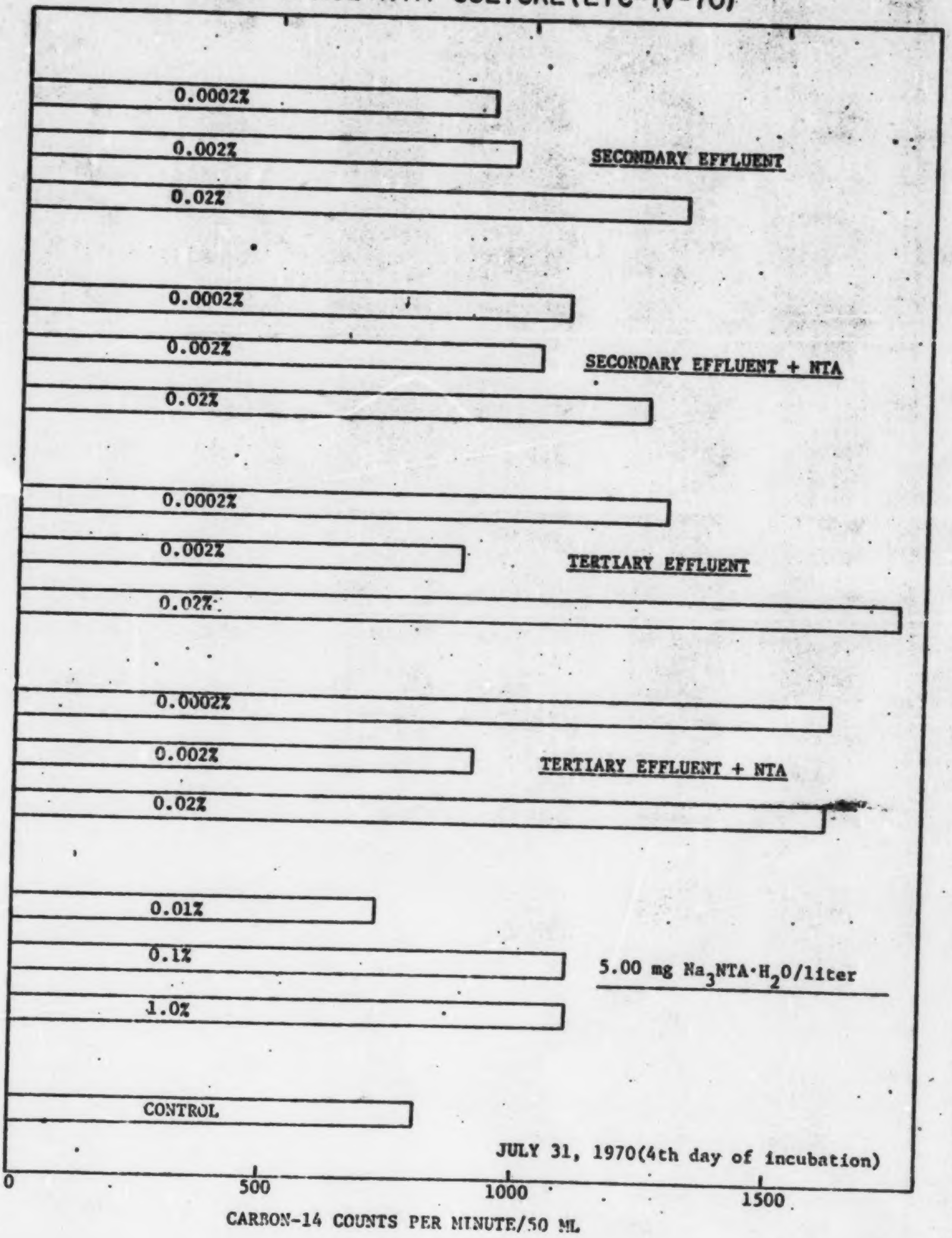


FIGURE 4

LAKE TAHOE NTA CULTURE (LTC-IV-70)



JULY 31, 1970(4th day of incubation)

FIGURE 5

LAKE TAHOE NTA CULTURE (LTC-V-70)

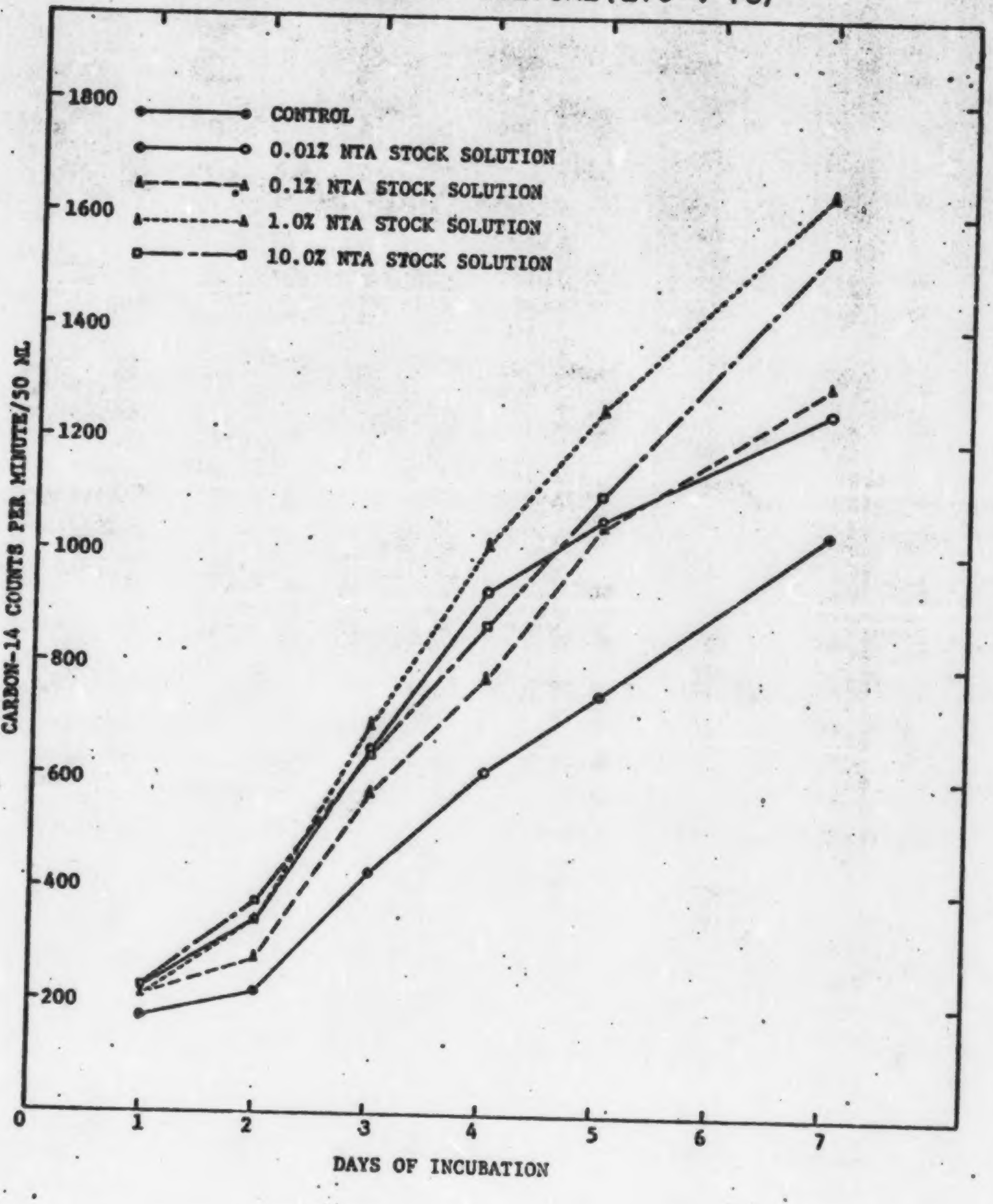


FIGURE 6

LAKE TAHOE NTA CULTURE (LTC-V-70)

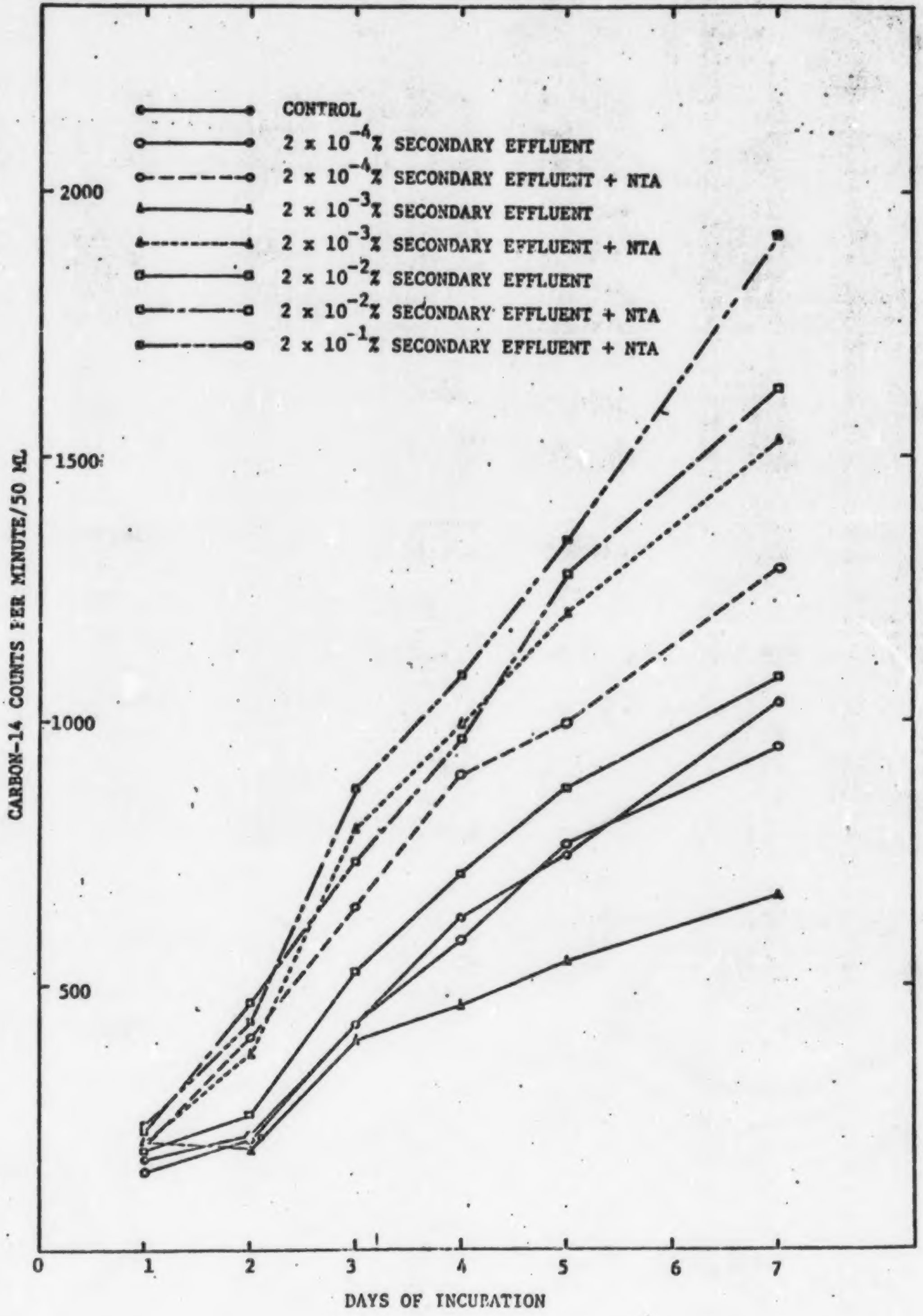


FIGURE 7

LAKE TAHOE NTA CULTURE (LTC-V-70)

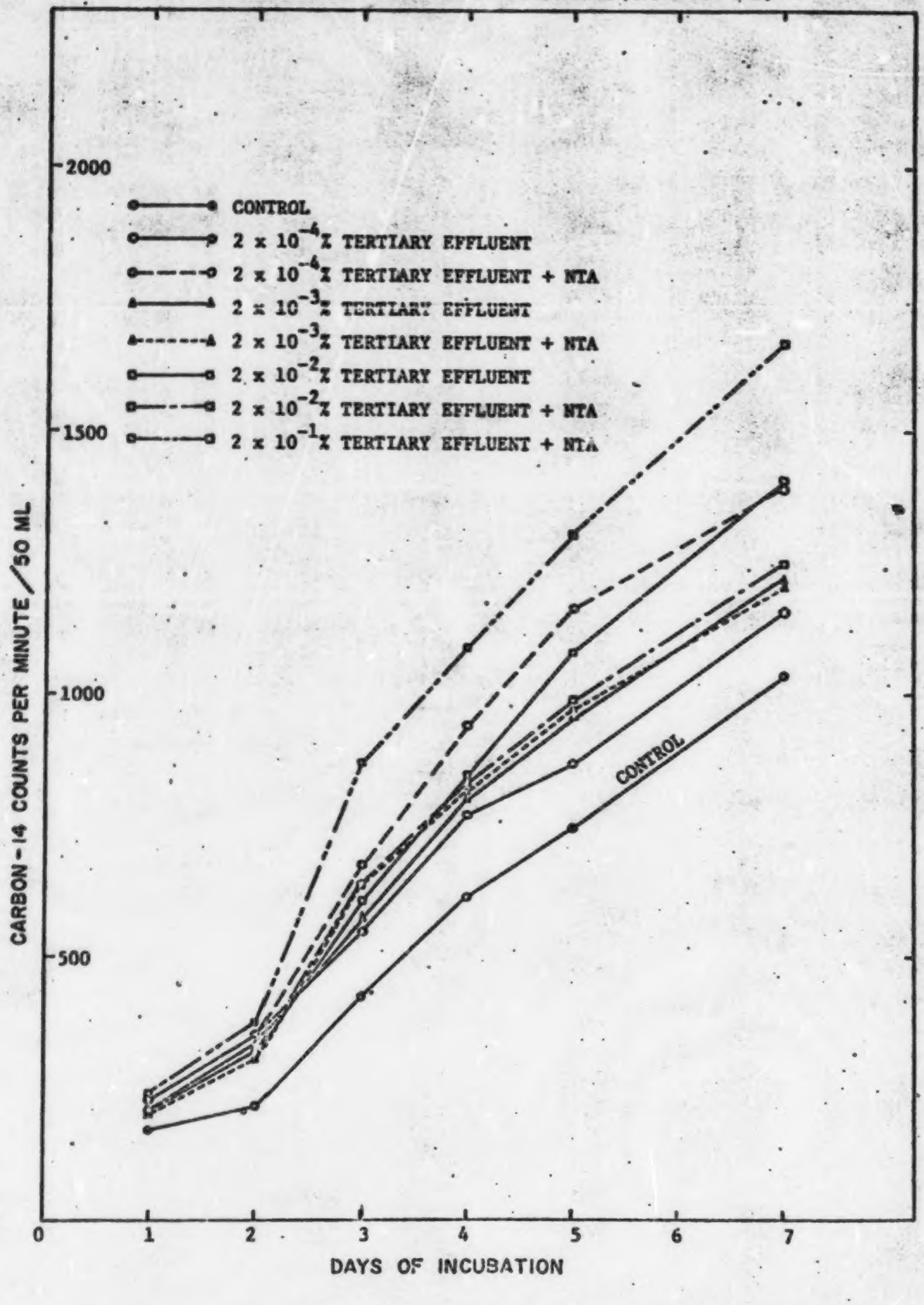


FIGURE 8

LAKE TAHOE NTA CULTURE (LTC-V-70).

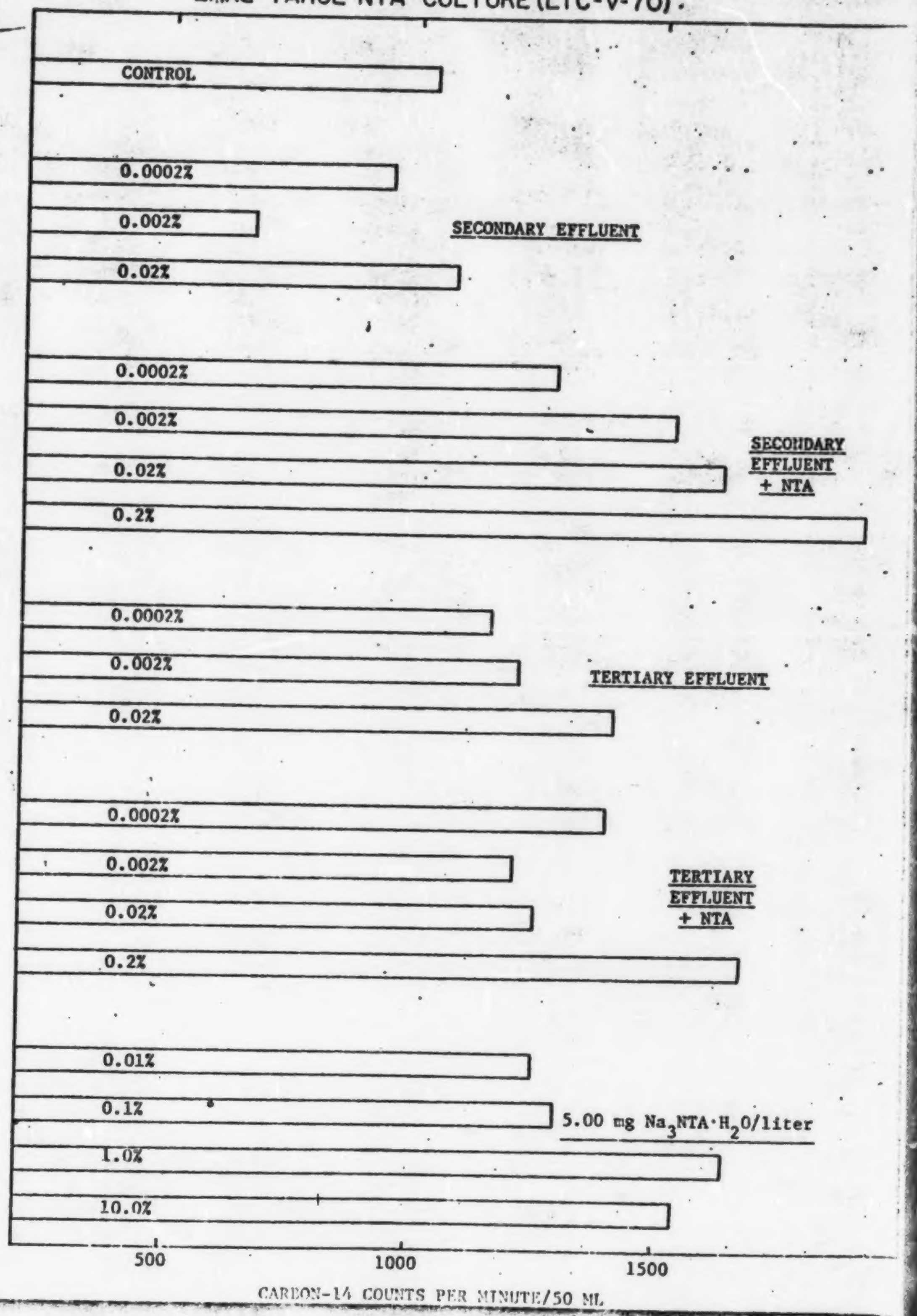


FIGURE 9

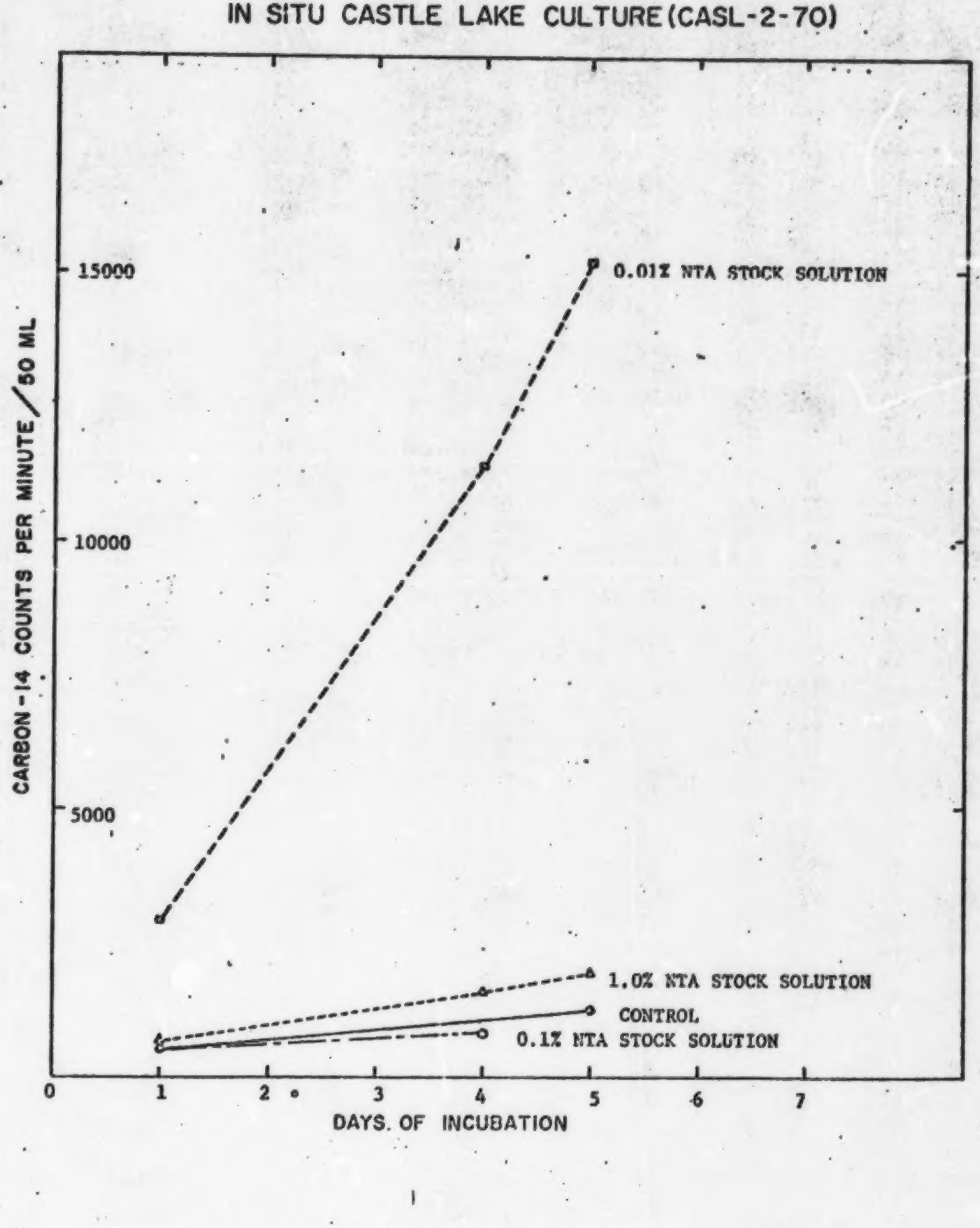

IN SITU CASTLE LAKE CULTURE (CASL-2-70)
CARBON-14 COUNTS PER MINUTE / 50 ML
15000
10000
5000
0
1
2
3
4
5
6
7
DAYS. OF INCUBATION
0.01% NTA STOCK SOLUTION
1.0% NTA STOCK SOLUTION
CONTROL
0.1% NTA STOCK SOLUTION

FIGURE 10

IN SITU CASTLE LAKE CULTURE(CASL-2-70)

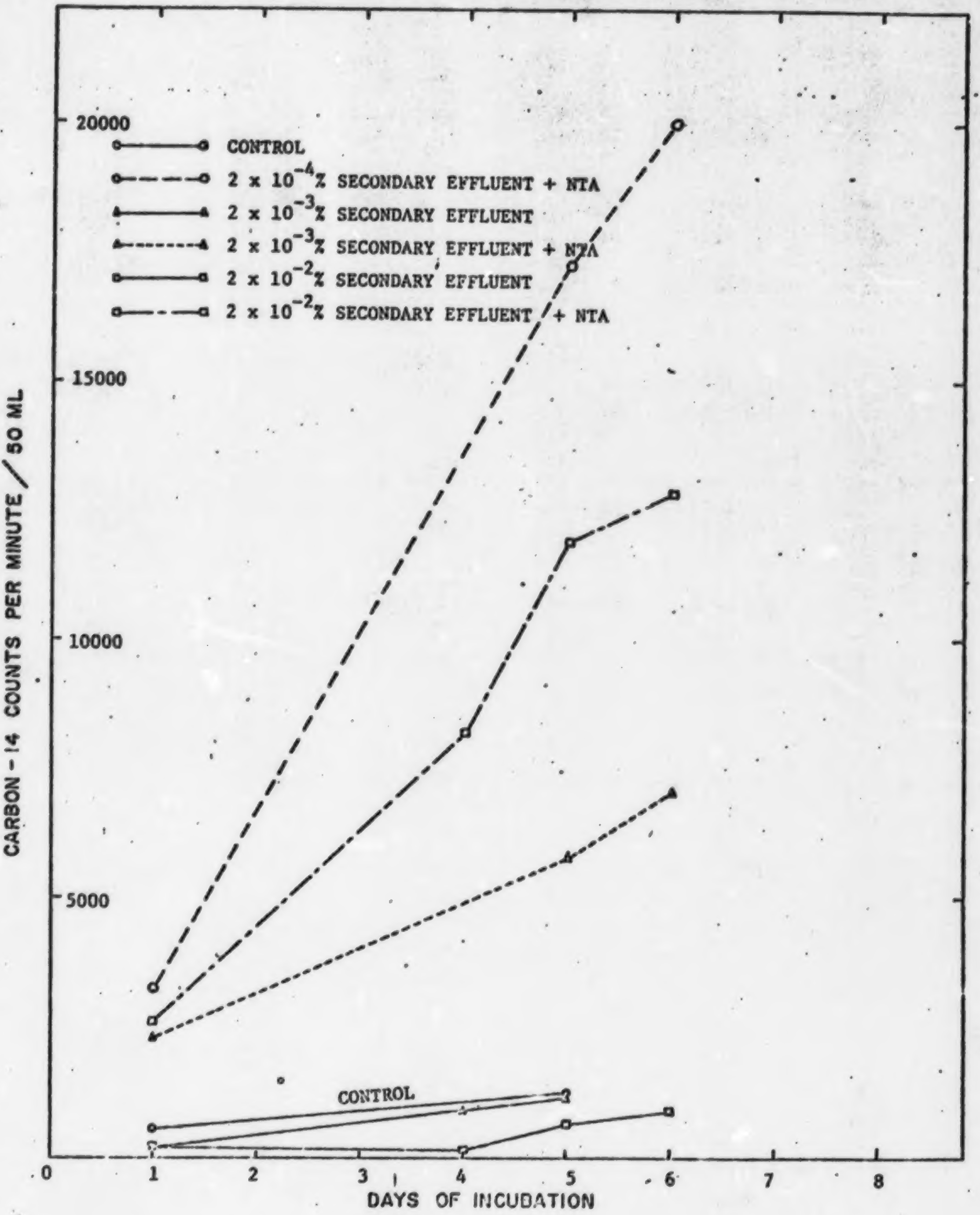


FIGURE 11

IN SITU CASTLE LAKE CULTURE (CASL-2-70)

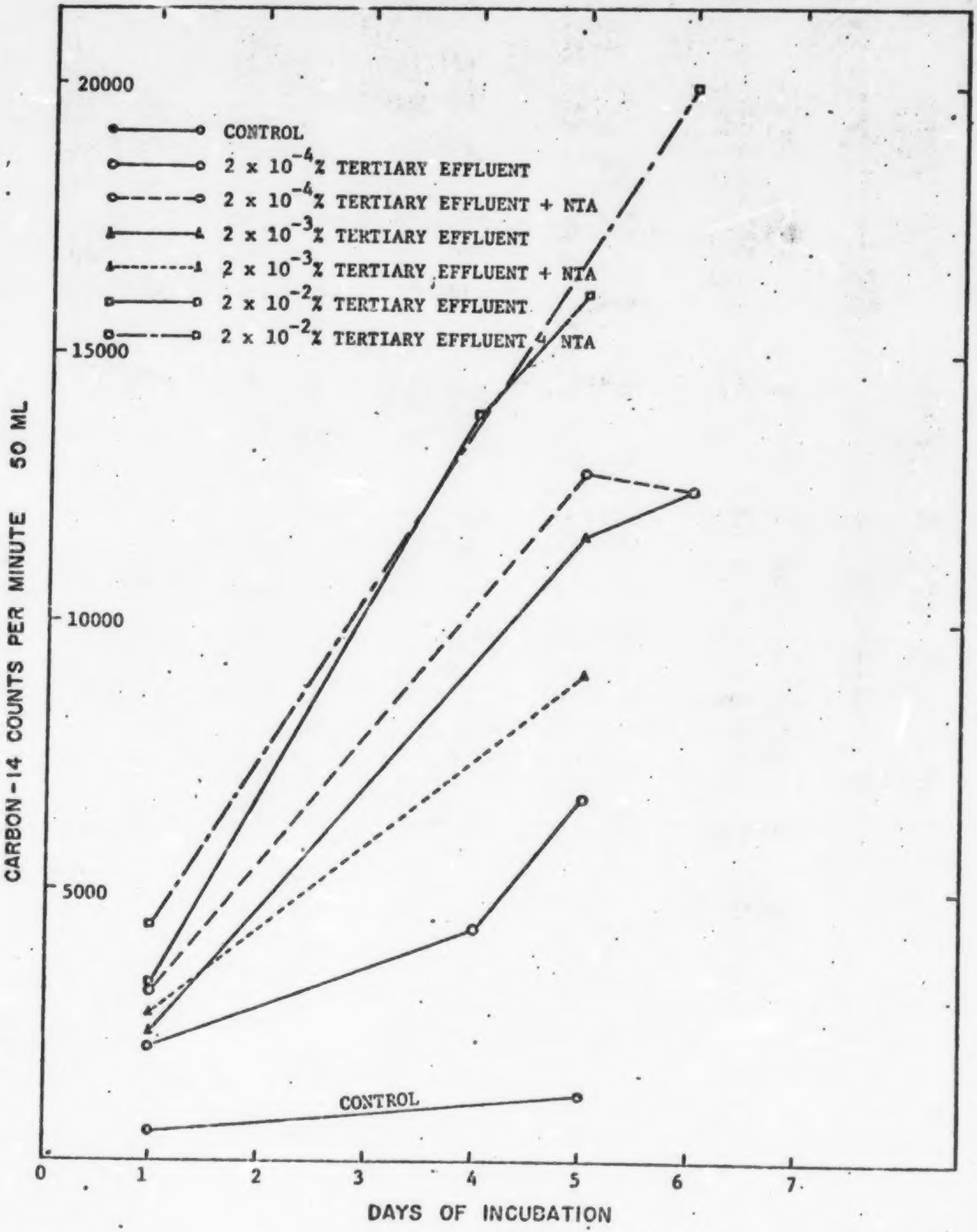


FIGURE 12

CASTLE LAKE NTA CULTURE (CASL-1-70)

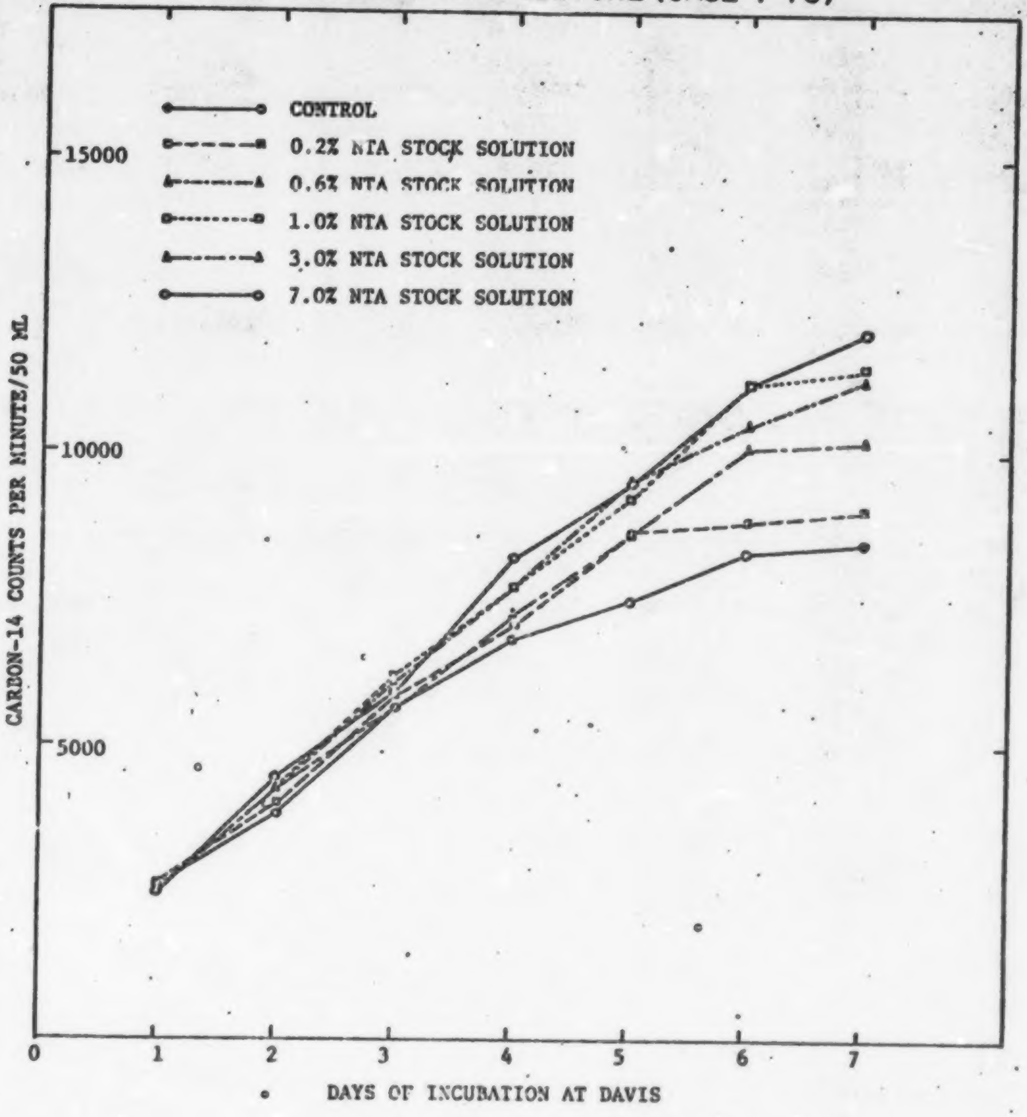


FIGURE 13

CASTLE LAKE NTA CULTURE (CASL-1-70)

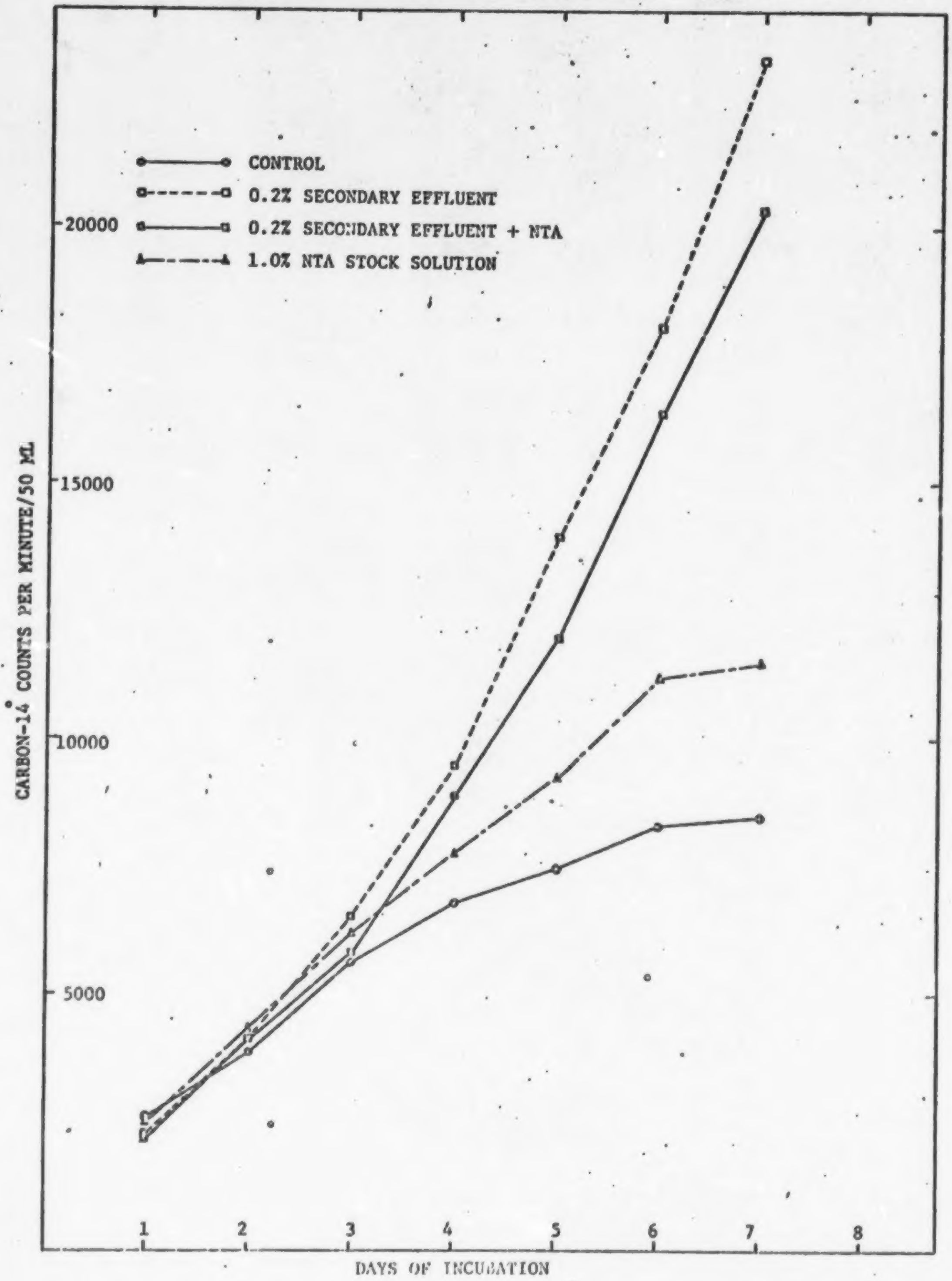


FIGURE 14

CASTLE LAKE NTA CULTURE (CASL-1-70)

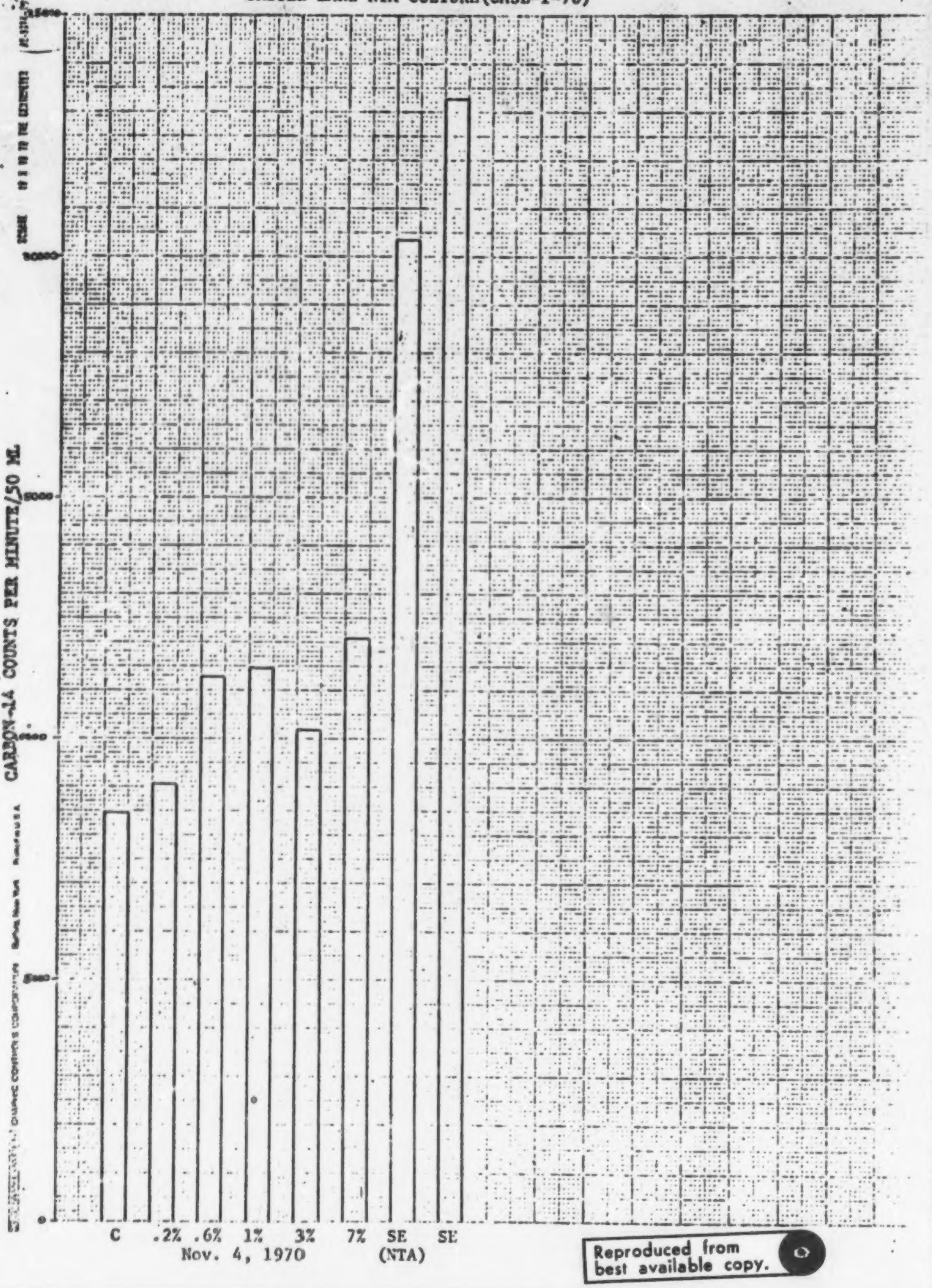


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FIGURE 15

CLEAR LAKE NTA CULTURE (CLC-1-70)

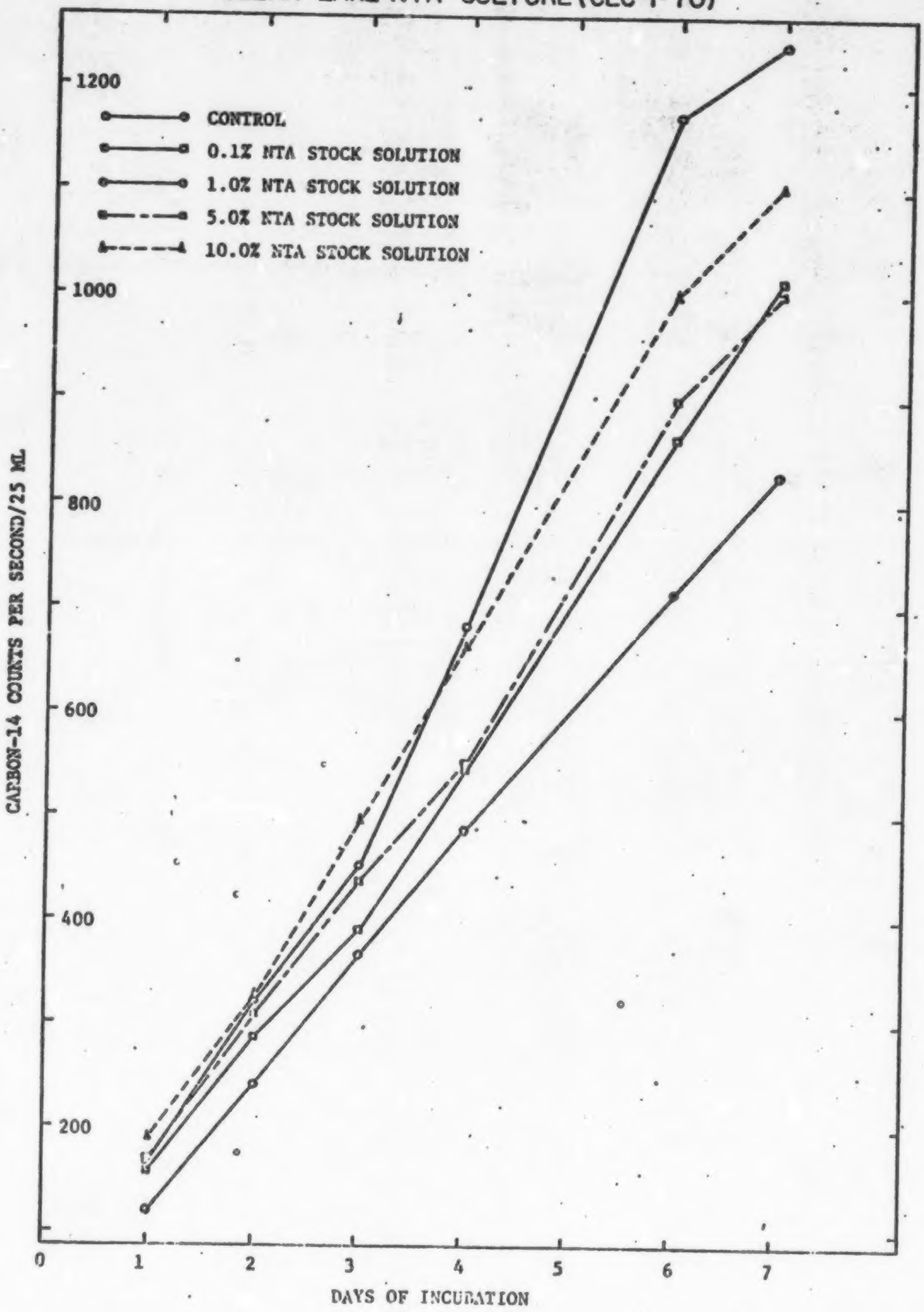

FIGURE 16

CLEAR LAKE NTA CULTURE (CLC-1-70)

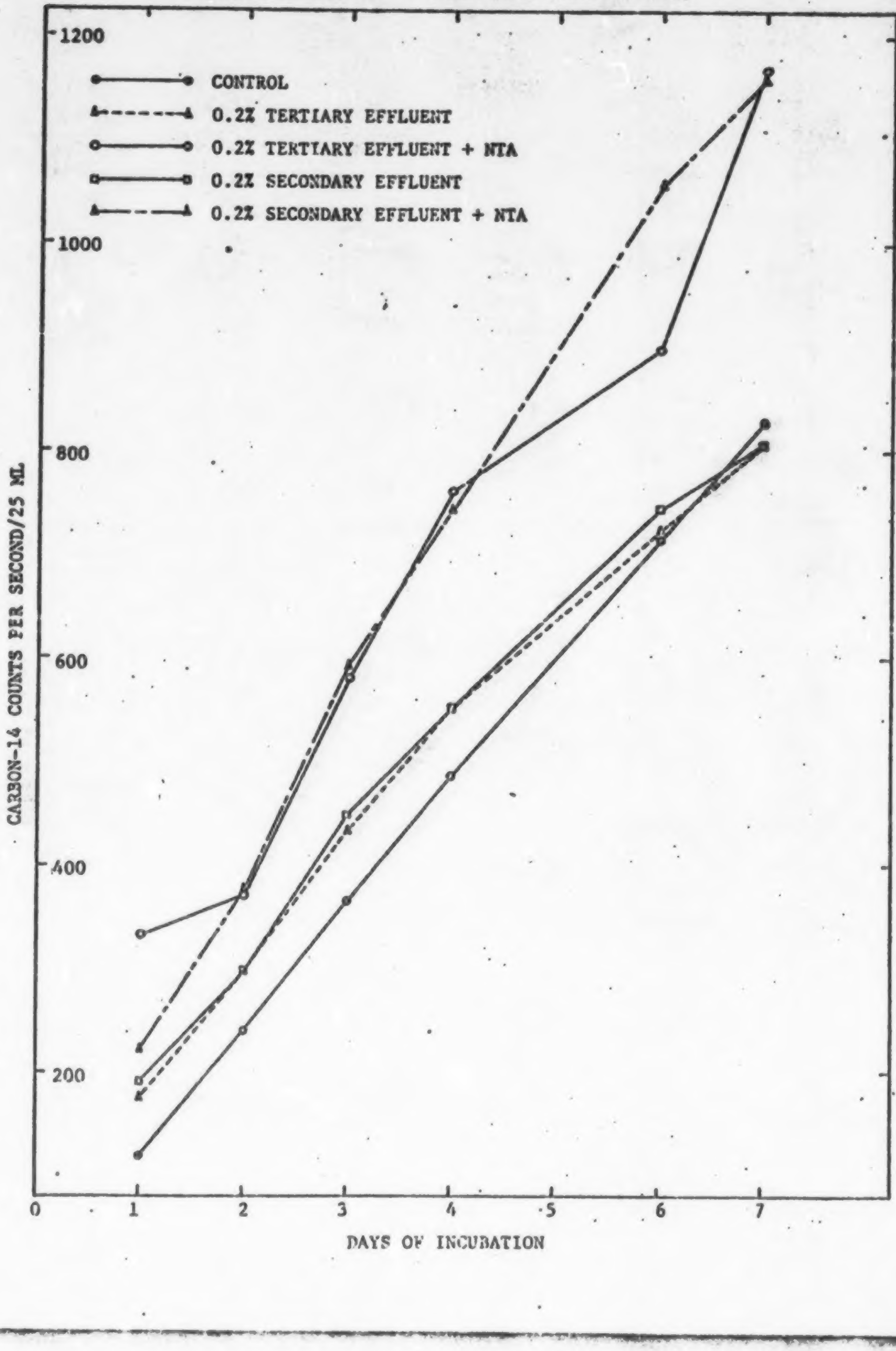

FIGURE 17

CLEAR LAKE NTA CULTURE (CLC-2-70)

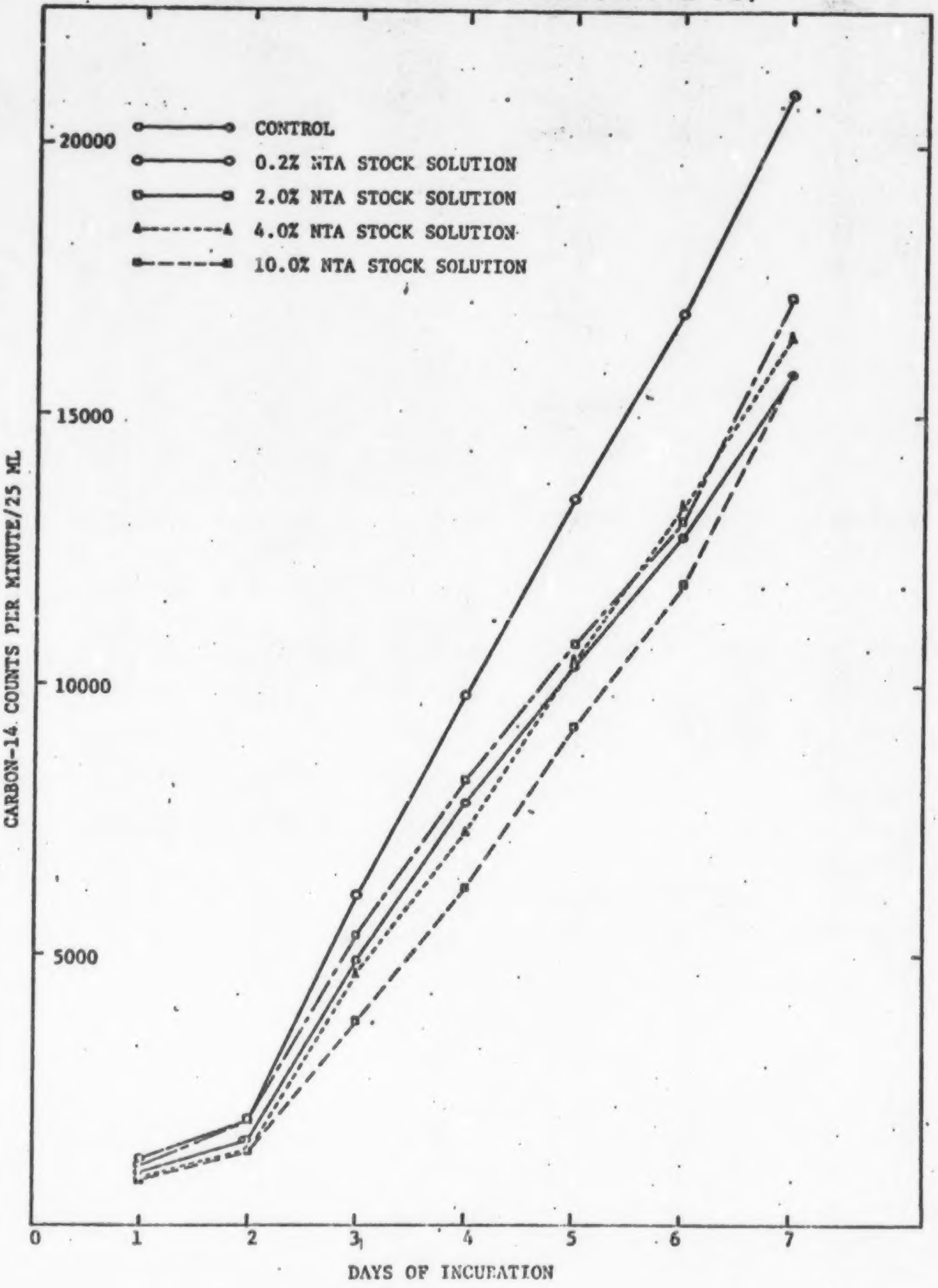

FIGURE 18

CLEAR LAKE NTA CULTURE (CLC-2-70)

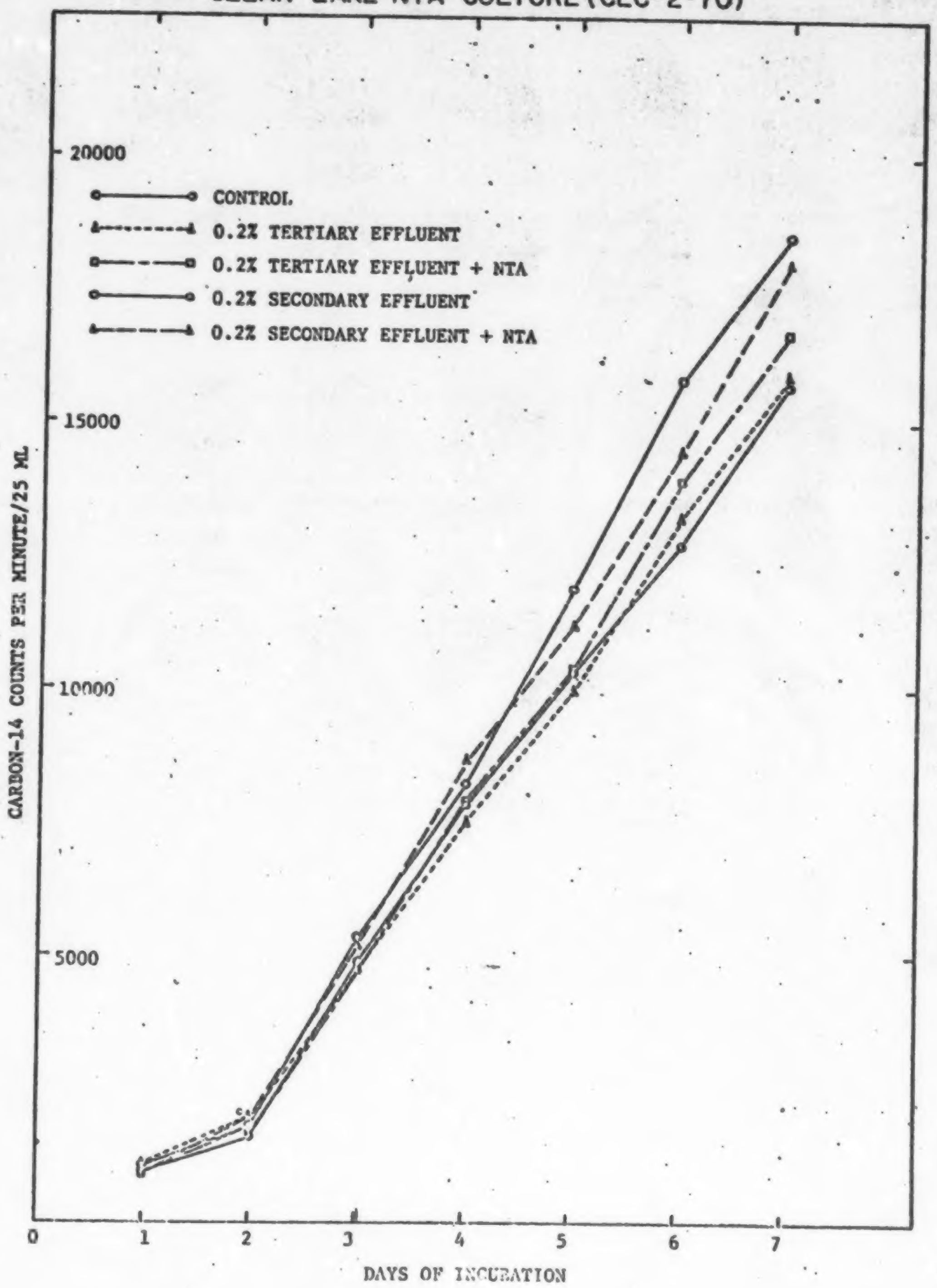

FIGURE 19

CLEAR LAKE NTA CULTURE #2(CLC-2-70)

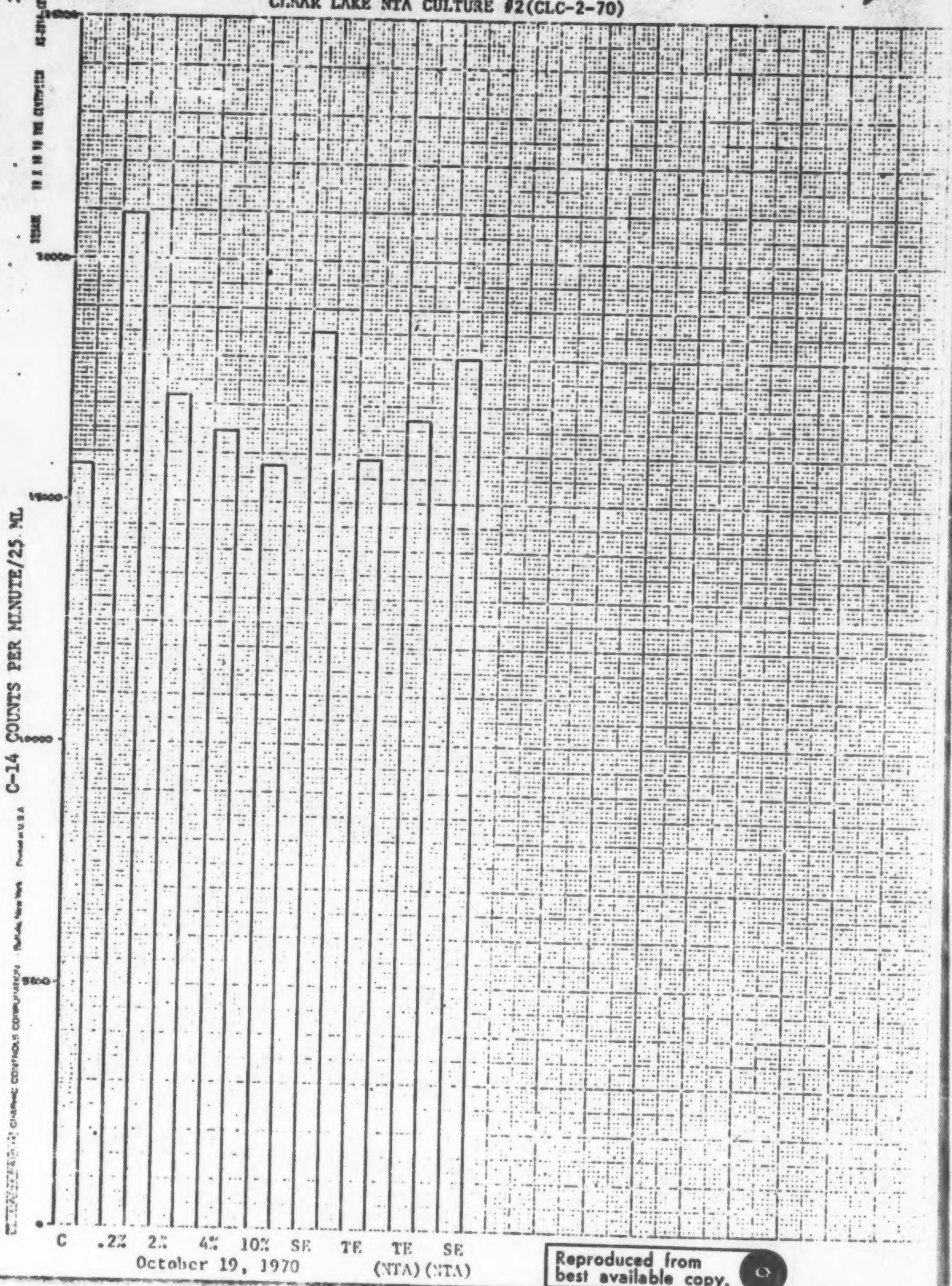

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FIGURE [illegible]

CLEAR LAKE NTA CULTURE (3CLC-3-70)

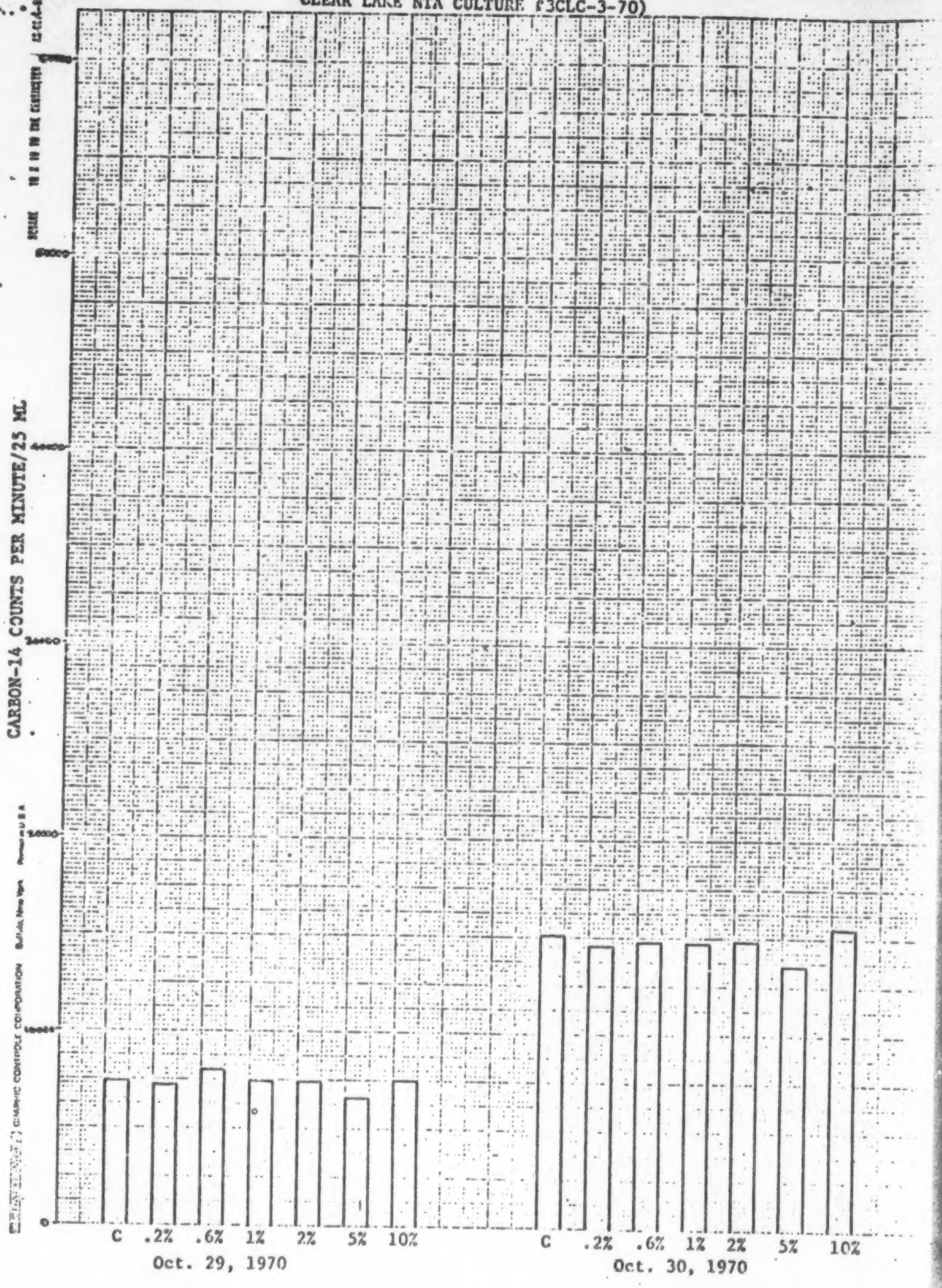

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FIGURE 21

CLEAR LAKE NTA CULTURE #3(CLC-3-70)

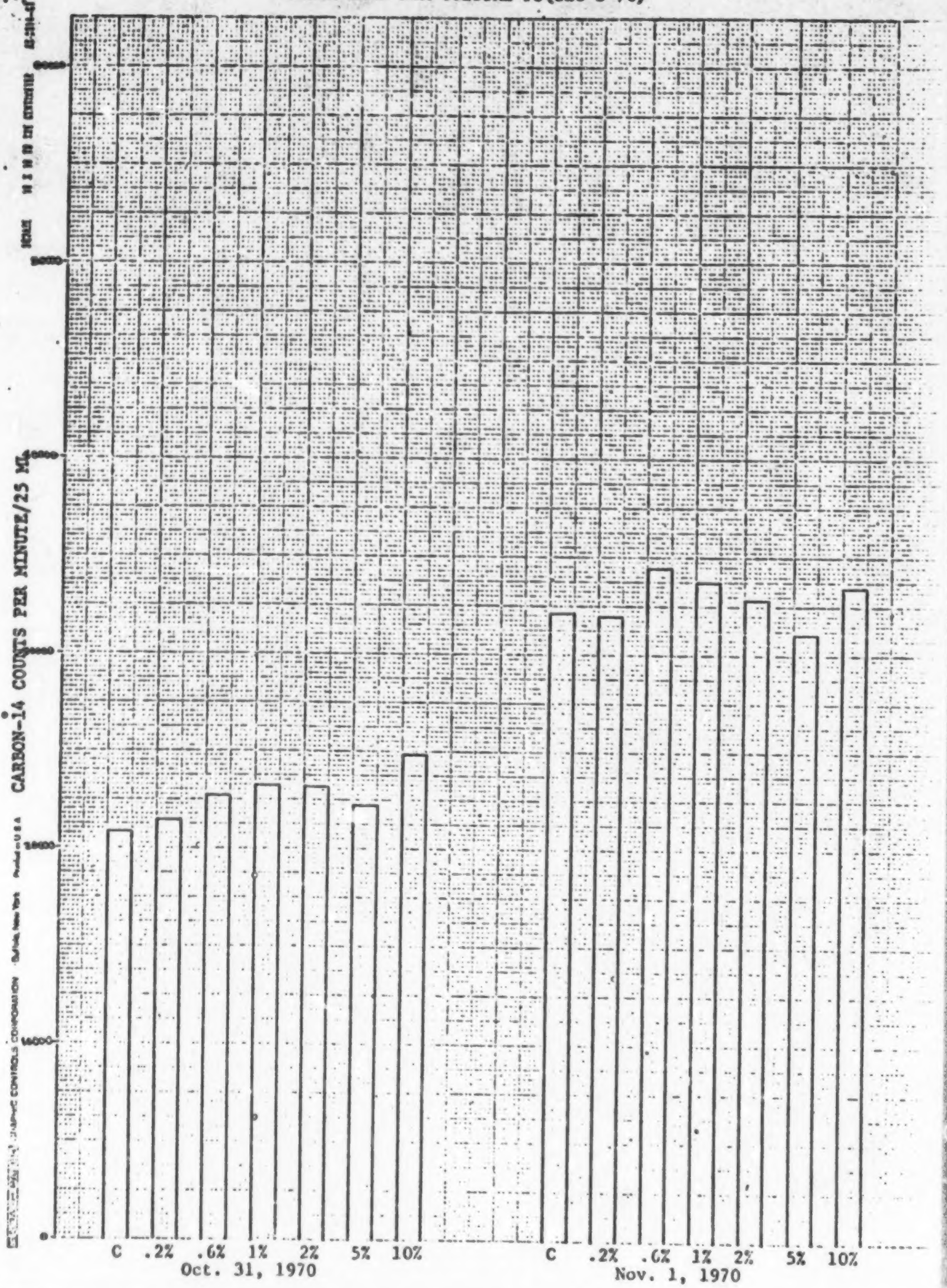

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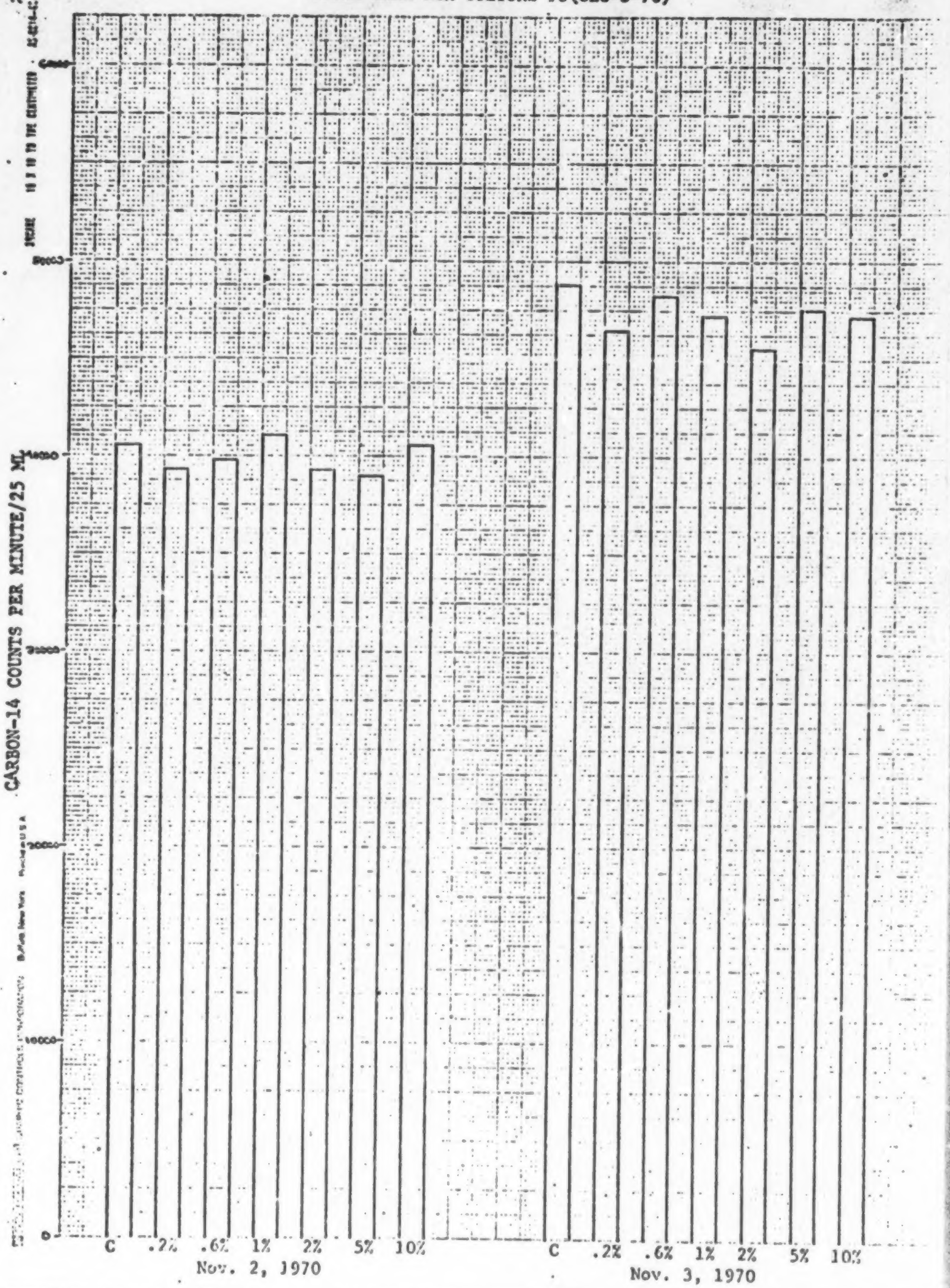
FIGURE 22
CLEAR LAKE NTA CULTURE #3(CLC-3-70)



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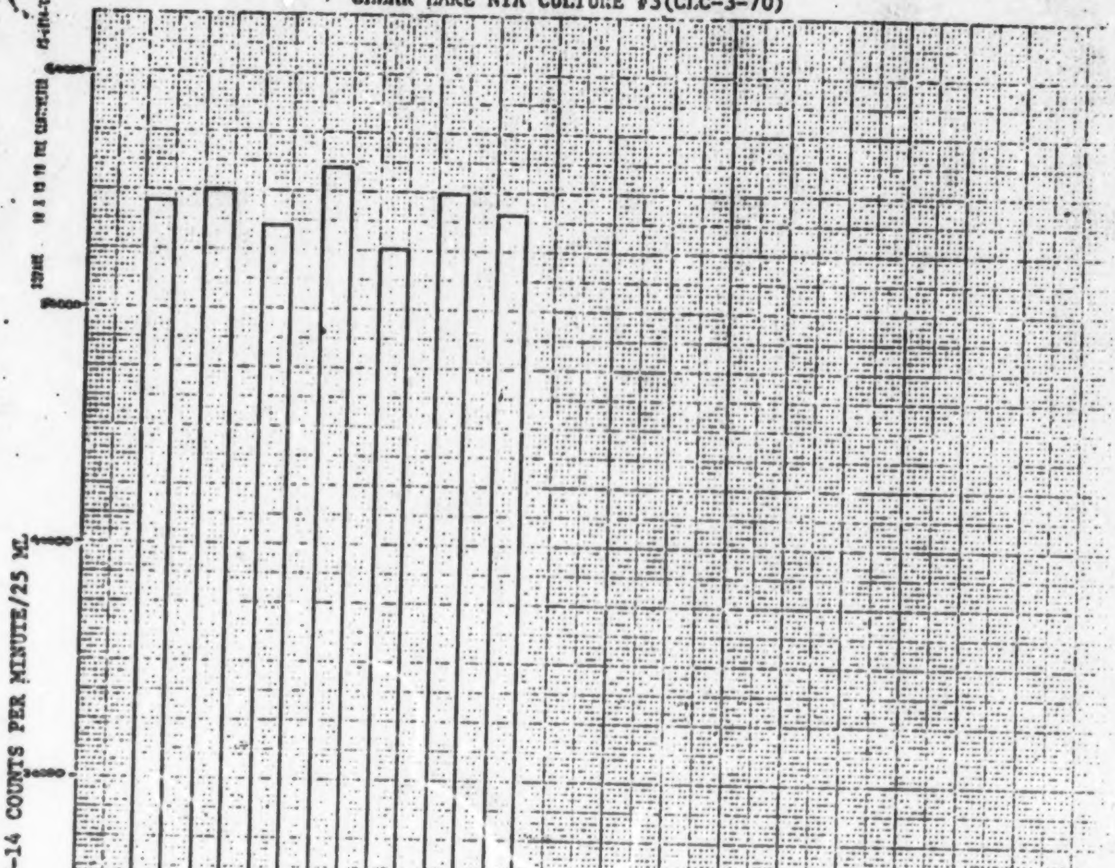

FIGURE 23
CLEAR LAKE NTA CULTURE #3(CLC-3-70)

Nov. 4, 1970

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APPENDIX C

University of North Carolina Report
"Influence of Nitrilotriacetic Acid (NTA) on
Eutrophication: *In situ* and Batch Assays."

Appendix C

THOMAS & MALONEY

Interim Report # 11

Provisional Algal Assay Procedure
FWQA Project Number 16010 DQT

INFLUENCE OF NITRILOTRIACETATE (NTA) ON EUTROPHICATION IN SITU AND BATCH-ASSAYS

Transmitted to:
T. E. Maloney, Project Officer
National Eutrophication Research Program
Pacific Northwest Water Laboratory
Corvallis, Oregon
November 13, 1970

Research Staff

Gail Marshall - Report Supervisor

Robert Drye

Technical Staff

Candace Gibbin
Thomas Ronman
Sandra Smith

[illegible]

Project Director

Charles M. Weiss - Report Supervisor

Department of Environmental Sciences and Engineering
School of Public Health
University of North Carolina at Chapel Hill

Summary

The potential of nitrilotriacetate (NTA) to act as an algal growth stimulant was evaluated by in situ Carbon-14 productivity determinations and batch assay according to the PAAP procedure. The NTA in pure chemical form as well as following passage through secondary and tertiary sewage treatment was tested in comparison with nitrogen and carbon provided from other chemical sources in amounts approximately equivalent to that found in the NTA molecule. In addition to untreated controls, the lake waters and NAAM media (0.3 PAAP media of October 1969), one test media included a phosphorus addition to the lake waters.

The in situ and batch assays were made on three North Carolina impoundments over the period of July to October 1970. Each lake water was used for four test series, of the in situ and batch assays respectively. The in situ experiments to determine effects of NTA on productivity as determined by carbon-14 uptake were of four hour duration and the sets of light-dark bottles were hung at a depth which provided 50% of surface illumination. Batch assays used 0.45 μ membrane filtered water reseeded with *Selenastrum capricornutum* at 10^3 cells/ml. Growth responses in the various test media were followed by cell counts through 21 days incubation at 24°C and 400 f.c. on an oscillatory shaker at 110 oscillations/min. Dry weight of cell mass formed and carbon synthesized were also determined at the end of the 21 day incubation period. Changes in pH of the media were also followed through the growth period.

Evaluation of the in situ productivity results found that the presence of NTA either in pure form or following passage through some level of sewage treatment produced no change in productivity, carbon fixed (mg/l-hr^{-1}), that exceeded the 90% confidence limits of the untreated lake water controls. The only materials added to the lake water which resulted in productivity values that were outside the confidence limits of the controls were the Na and K carbonates and these on

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several tests resulted in productivity levels that were lower than the controls.

The batch assays of NTA as an algal growth stimulant essentially confirmed the results of the productivity tests. No medium containing NTA either in pure form or after passage through secondary or tertiary sewage treatment levels resulted in growth, by any parameter used, which exceeded that of the specific control in any significant manner. The phosphorus supplement used for comparative purposes in this test, even though used at 1/5 the concentration originally specified resulted, in nearly every assay series, in growth greater than the control the nitrogen supplement or the NTA addition.

One interesting aspect of the productivity determinations was the finding that if the productivity of the lake controls were compared to the total phytoplankton count of the sample and total insolation for the period of exposure there appeared to be indicated an optimum cell concentration and insolation for the peak productivity values.

Introduction

Nitrilotriacetate (NTA) has been proposed as a substitute for phosphates in detergents. In light of the quantities of such materials introduced via laundry wastewater into rivers and lakes, the substitution of NTA for phosphates clearly indicated that an evaluation of this nitrogen-rich material and its possible eutrophication potential was a high national priority. As part of an ongoing research project with the Federal Water Quality Administration (16010 DQT) a supplement to the 1970 budget was made to fund a series of experiments which would provide information that would permit an evaluation of the effect of NTA on algal growth. The basic investigation was to compare in situ carbon-14 productivity determinations in several lakes with PAAP batch culture assays. This investigation was initiated on July 1, 1970 and completed early in November 1970.

Methodology

The effects of NTA were to be evaluated not only as to the effect on algal growth of the pure chemical but as well as the nature of NTA following sewage treatment at secondary and tertiary levels. In addition, approximate equivalents of nitrogen and carbon as found in the NTA molecule as well as phosphorus supplements to the test waters were to be evaluated. Untreated lake waters were to serve as controls. The NAAM medium (0.3 PAAP medium, October 1969) was to serve as the reference medium for the batch culture tests. The composition and identification code of the various media that were used in this NTA evaluation are listed in Table 1. The use of numerical designations for these various combinations of water, NTA, sewage, etc., will be used throughout this report in the various tabulations and figures illustrating the results. Thus, Table 1 becomes the basic reference page for media composition as well as concentrations of N, P, and C used as media supplements.

TABLE 1

Composition and Identification Code of Various Media Used in NTA Evaluation

Medium	Symbol	Media (Value in parentheses is amount added to cultures.)	N, P and/or C,* Concentratio As Added to Lake Water N (mg/1)	P (mg/1)	C (mg/1)
1	○	Lake water + NTA (5 mg/1)	0.255	--	0.00
2	◉	Lake water + sec. effluent fed with primary effluent containing 5 mg/1 NTA - amount of effluent added = 2% culture volume	0.410	0.248	0.25
3	△	Lake water + tertiary effluent resulting from NTA treated secondary effluent - amount of effluent added = 2% culture volume	0.380	0.012	0.56
4	▲	Lake water + secondary effluent - amount effluent added = 2% culture volume	0.356	0.236	0.36
5	□	Lake water + tertiary effluent- amount effluent added = 2% culture volume	0.380	0.002	0.58
6	▣	Lake water + $NaNO_3$	0.365	--	--
7	▽	Lake water + $NaHCO_3$	--	--	1.89
8	▼	Lake water + $KHCO_3$	--	--	6.0
9	⌖	Lake water + K_2HPO_4	--	0.01	--
10	⌖	Lake water - No additions	--	--	--
11	✡	NAAM Medium - (See Appendix for components)			
12	✡	Lake water + K_2HPO_4	--	0.05	--

*Inorganic C only.

The three lakes used in this test are all representative impoundments of North Carolina. Their physical characteristics are described in Table 2. University Lake is the water supply impoundment of Chapel Hill and is owned and operated by the University of North Carolina. Lake Michie is the water supply impoundment of the city of Durham and Lake James is a hydroelectric impoundment of the Duke Power Company. The three lakes provide an interesting comparison in that they range from a very small body of water (University Lake) to one of respectable size, Lake James. The latter, high in the foothills of the Appalachian Mountains, drains an area that is heavily wooded and in many respects little changed from the original forested mountain slopes. Because of an unusually dry period during this evaluation series, extreme drawdown conditions in Lake James as well as in the water supply impoundments were noted.

The chemical characteristics of these lakes on the dates sampled is presented in Table 3. The determinations for the nitrogen and phosphorus species were made on Technicon Autoanalyzers, the alkalinity was determined by titration, and the hardness computed (Standard Methods) from calcium, magnesium, iron and manganese concentrations as determined on a atomic adsorption spectrophotometer. pH was determined in the field with a portable pH meter.

Nitrogen as indicated by the quantity of NH_3- or NO_3-N was, in nearly every instance, in each lake extremely low and was probably growth limiting. It should be noted that in the period of this study University Lake was undergoing an experimental investigation on destratification and the relatively high ammonia nitrogen levels may probably be due to the artificial stirring. The chemical characteristics of the lakes as seen on the four dates of sampling may best be summarized as follows: In University Lake Kjeldahl nitrogen was relatively uniform throughout the period of testing with a slight increase on the last date of sample. In Lake Michie Kjeldahl -N was about the same for three samples but dropped on the last sample. Lake James in contrast showed a systematic decrease on each

TABLE 2

Physical Characteristics of Three Lakes Used in NTA Evaluation

	University Lake	Lake Michie	Lake James
Location	4 miles west of Chapel Hill, N.C. Orange County	12 miles north Durham, N. C. Durham County	8 miles west Morganton, N.C. Burke, McDonald Counties
Year Completed	1932	1926	1919
Length - miles	1.56	5	14.36
Surface Area - acres	200	540	6510
Shoreline Length - miles	4.85	10.3	150
Depth at Dam - feet	30	80	110
Storage Volume - acre feet	1872	138,100	300,000
Average Retention Time - Days	—	—	228

TABLE 3

Chemical Characteristics of Lake Waters As Used For In Situ Productivity Evaluation Of NTA and Other Materials, July - October 1970

	University Lake				Lake Michie				Lake James			
	July 16	Aug 13	Sept 9	Sept 30	July 23	Aug 19	Sept 16	Oct 8	Aug 6	Aug 27	Sept 24	Oct 15
Nitrogen - mg/l												
NH_3-N	.015	.04	.17	.22	.07	.01	< .01	< .01	.01	< .01	< .01	< .01
NO_3-N	.02	.024	.010	.030	.02	.001	.004	.007	.004	.003	.015	.006
Kjeldahl-N	.20	.34	.25	.42	.22	.30	.22	.14	.61	.30	.20	.15
Phosphorus - mg/l												
Ortho-P	.006	.045	.001	---	.003	.025	.002	.005	.009	.038	.009	.002
Total-P	.038	.096	.005	.016	.038	.035	.003	.011	.05	.033	.012	.006
Alkalinity mg/l $CaCO_3$	25.5	24.0	24.0	25.5	9.5	12.0	12.0	13.5	18.0	15.0	18.0	17.0
Hardness mg/l $CaCO_3$												
Filtered	15.94	14.72	13.36	18.76	9.41	10.13	14.04	10.17	8.84	8.50	9.56	6.34
Raw	---	---	18.16	19.82	---	---	13.98	12.07	---	8.93	9.85	7.32
pH	7.0	7.7	6.8	6.8	6.8	7.0	7.2	6.6	8.6	8.6	9.0	8.8

successive date of sampling. Total phosphorus varied considerably, although always at extremely low levels with no systematic changes indicated. The alkalinity of University Lake was about twice the level of Lake Michie with Lake James intermediate between the two. In turn, the hardness determination was highest for University Lake, intermediate for Lake Michie and lowest for Lake James, although all three lakes can be be classified as quite soft. In several instances the hardness determination was made on both the filtered as well as raw water samples, the latter nearly always being slightly higher than the former. The pH of University Lake and Lake Michie was close to neutral on all occasions whereas Lake James was distinctly and markedly more alkaline, being at 8.6 or higher on every occasion. This high alkalinity can only be attributed to biological activity.

As previously noted University Lake was undergoing an experimental study on destratification and the vertical temperature distribution as shown in Table 4 illustrates the uniformity of temperature in the upper 10 feet. Lake Michie had a normal pattern of temperature distribution for a lake of this latitude but was showing some degree of mixing by the last sample in October, as was Lake James. The Secchi disc readings for the three lakes, also shown on Table 4, describe University Lake as having on the average a somewhat shallower Secchi depth than Lake Michie or Lake James, the latter being somewhat clearer on the average for the four samples. The in situ productivity experiments were hung on the date of test at a depth to which 50% of the surface illumination penetrated as determined with an underwater photometer. By this measurement Lake Michie and University Lake were approximately of the same clarity whereas Lake James was somewhat clearer.

In order to provide a basis for comparison of the productivity of the untreated lake waters the solar energy input to the carbon converting system should be determined. The quality of light in any one day will vary considerably with the passage of clouds or the particular meteorological conditions for the date. A

TABLE 4

Vertical Temperature Distribution and Light Penetration Characteristics of The Lakes Used in In Situ C-14 Productivity Determinations for NTA Evaluation

July - October 1970

Depth Feet	University Lake				Lake Michie				Lake James			
	July 16	Aug 13	Sept 9	Sept 30	July 28	Aug 19	Sept 16	Oct 8	Aug 6	Aug 27	Sept 24	Oct 15
0	27.8	26.2	26.9	22.6	28.8	27.2	26.3	22.6	29.3	26.0	31.8	22.1
1	27.7	26.1	27.0	23.1	28.8	27.2	26.3	22.0	29.3	26.0	31.4	2 .0
2	27.7	26.1	27.0	23.1	28.8	27.2	26.3	21.7	29.0	26.0	31.3	21.8
3	27.6	26.0	27.0	23.1	28.4	27.2	26.3	21.7	29.0	26.0	31.5	21.4
4	27.5	25.9	27.0	23.1	28.0	27.2	26.2	21.5	28.9	26.0	31.0	21.2
5	27.4	25.6	27.0	23.1	27.0	27.0	26.1	21.2	28.9	26.0	30.5	21.2
6	27.4	25.3	27.0	23.1	26.0	26.5	26.1	21.0	--	26.0	30.0	21.0
7	27.4	25.2	27.0	23.1	25.5	26.0	26.1	21.0	--	--	29.8	--
8	27.4	25.0	27.0	23.1	25.0	24.8	26.1	21.0	--	--	29.5	--
9	27.3	24.9	27.0	23.8	24.5	--	26.1	21.0	--	--	29.1	--
10	27.3	24.5	27.0	24.0	24.0	--	26.1	21.0	--	--	28.9	--
Secchi Depth	3.3	2.75	3.3	2.5	2.5	5.0	5.0	2.4	4.0	4.75	4.5	5.0
Depth - 50% of Surface Light	1.0	1.0	0.5	.75	0.7	1.5	1.0	0.75	1.25	1.25	2.5	1.5
Total Insolation 4 hr Test cal/cm^2	170.63	129.97	130.74	93.3	129.55	99.59	128.81	69.60	99.37	134.9	76.00	68.83

solar energy integrating meter* was available and through the linkage of its output to a strip recorder the insolation for the period of test was recorded. From this recording the total energy falling on the surface of the lake for the 4 hour test period was calculated as calories/cm^2. As shown in Table 4 insolation varied considerably from date to date and must be used in comparing the relative level of productivity as measured on any one date. As will be developed in the section on results, a figure has been prepared for the control samples (no added materials) which relates productivity, insolation and total phytoplankton in the test samples.

Critical to the comparison of carbon-14 productivity levels is the total number of phytoplankton in the sample as well as species characteristic of the sample. This analysis, within the time available for describing and defining the population characteristics for each sample, was carried out in the following manner. The raw water sample was concentrated using Utermohl sedimentation cylinders. The concentrated sample was prepared in a permanent mount according to the method of Dickman (1968). Counting was by the clump method, counting individual colonies and filament segments of 46u in length as single units. In the tabulation of the population characteristics of each sample, all species representing at least 5% of the total were recorded (Table 5). In each sample, in addition to those species above the 5% level, other scarcer species have been noted only as part of the total number of species for that date. The September 30 sample from University Lake was unusually barren of detectable plankters although still showing a chlorophyll extractable fraction comparable to previous samples. The productivity determination on the control sample for this date was also lowest of the four determinations for this body of water. The unusual bloom of Chlamydomonas on Lake Michie on October 8 was reflected in the high chlorophyll extraction but the productivity of lake control was quite low possibly due to the

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* St. Paul Scientific, Inc., St. Paul, Minnesota

TABLE 5

NTA EVALUATION

Plankton Analysis of Lake Samples Used in Productivity Determinations (cells/ml)

	University Lake				Lake Michie				Lake James			
Date	7/16	8/13	9/9	9/30	7/28	8/19	9/16	10/8	8/6	8/27	9/24	10/15
Total Count	770	1207	1769	[redacted]	798	465	916	7352	4203	1200	1138	853
Total Species	14	11	19	--	10	3	7	7	7	8	4	7
Chlorophyta												
Volvocales												
Chlamydomonas					166			6832	201	62	69	146
Chloroccales												
Coelastrum												
Ankistrodesmus												
Scenedesmus												153
Crucigenia												
Euglenophyta												
Euglenales												
Lepocinclis	160											
Trachleomonas					146							
Pyrrhophyta												
Peridiniales												
Gonyaulax	49	312										
Glenodinium	83	111			166							
Cyanophyta												
Chroococcales												
Microcystis		201										
Merismopedia			798									
Oscillatoriales												
Anabaena		264				76	867		3579	926	853	305
Unknown filamentous forms	69		111			305		243				
Unknown flagellates		118	257									111
Chlorophyll Extract-Klett Units	16	8	9	8	--	5	10	17	6	6	3	9

Numbers shown are species counts that were at least 5% of total count.

University Lake sample of 9/30 extremely sparse.

low insolation for the exposure period.

The carbon-14 uptake experiments were carried out in duplicate clear bottles and one dark bottle for each test medium. At the end of the four hour exposure in situ the samples were "killed" with Lugols solution and returned to the laboratory for filtration and counting. All steps and procedures were according to the PAAP document. Similarly, the water for batch assay followed the PAAP document, filtration on return to the laboratory, reseeding, after addition of the supplement spike, with 10^3 cells/ml of Selenastrum capricornutum. Incubation was at 24°C under 400 f.c. on an oscillatory shaker at 110 cycles per minute.

Results

In Situ Productivity

The evaluation of the various additions to the lake waters, as outlined in Table 1 on in situ productivity and growth in batch assay, is summarized in a series of tables and figures. Table 6 presents the results of in situ productivity as carbon fixed mg/l per hour. If the lake control (No. 10) is used as the reference value on each date of sample it would appear at a glance that differences between other media did exist and the differences in many instances appear to possibly be significant. If comparison is made just between the control sample and No. 1 medium (NTA added at 5 mg/l), it is seen that in the University Lake series three of the four samples, September 8 being the exception, the addition of NTA appeared to produce a higher rate of carbon fixed than in the control. In Lake Michie, comparing the same two media on the four dates of sample, a consistent pattern was found throughout, the presence of pure NTA producing a higher level of productivty. In Lake James on one date the lake water and the NTA addition were about the same in carbon fixed (August 6). On another occasion, October 15, the addition of NTA produced a somewhat lower rate of carbon fixed and on the other two dates the NTA sample had a somewhat higher rate.

TABLE 6

Effect of NTA and Other Materials On *In Situ* Productivity of Lake Waters

Carbon Fixed - mg/l - hr x 10^2
July - October 1970

	University Lake				Lake Michie				Lake James			
	July 16	Aug 13	Sept 9	Sept 30	July 28	Aug 19	Sept 16	Oct 8	Aug 6	Aug 27	Sept 24	Oct 15
1. NTA - 5 mg/l	12.64	11.84*	7.08*	7.29*	9.86*	2.55	3.75*	4.75	7.13	3.42	15.49*	2.21
2. Secondary effluent fed primary containing 5 mg/l NTA	10.99	9.83	9.06*	5.79*	5.45	2.59	2.04*	1.71	6.94	3.92	11.57	5.78
3. Tertiary effluent fed with #2	9.72	11.35*	11.94	6.35*	7.19*	2.97	2.51	3.63	8.05	3.83	12.07	7.05*
4. Secondary effluent 2%	9.22	10.91*	11.33	4.98	5.86	1.98	2.71	1.79	7.35	3.99	11.86	6.38*
5. Tertiary effluent 2%	9.60	10.57*	10.59	4.58	7.96*	2.64	3.03	3.45	7.47	5.67	12.67*	4.96
6. N ($NaNO_3$) .365 mg/l	10.12	9.54	11.21	4.38	5.40	2.19	2.86	4.94	6.97	4.02	11.91	2.65
7. C ($NaHCO_3$) 1.89 mg/l	7.21	9.68	10.44	4.26	6.84*	2.58	2.95	2.84	7.93	4.38	11.40	4.62
8. C ($KHCO_3$) 6.0 mg/l	7.21	9.05	8.61*	4.53	4.03	1.83	2.55	2.99	6.37	3.19	9.16	3.71
9. P (K_2HPO_4) .01 mg/l	9.17	8.72	9.93	3.58	5.31	2.55	2.44	1.69	6.35	2.74	11.01	2.81
10. Lake Control	9.33	7.72	10.93	3.98	4.24	1.74	2.87	2.87	7.21	2.95	9.99	3.02
Standard Deviation	1.2819	.8809	.4584	.5324	.6948	.5222	.1865	.7155	.6810	.9693	.7361	.9718
Least Significant Difference	4.0388	2.7750	1.4440	1.6774	2.1890	1.6452	.5877	2.2540	2.1455	3.0538	2.3191	3.0614

*Samples that exceed the least significant difference at the .05 level compared to the Lake control.

To establish a valid basis for comparing the various carbon fixation rates with some degree of statistical confidence, the actual scintillation counts for the duplicates in each sample were used to calculate the 90% confidence limit for each test condition on each date. The determination of counts per minute was computed as an average of three successive counts for each particular sample, then the value for the two samples in each set treated as a pair and the confidence limit computed based on the variation from the average of this pair. These results are shown in Figures 1, 2 and 3 for each lake on the four sampling dates. With only two samples available for analysis the 90% confidence limit in some instances tends to be rather broad. However, in nearly all cases the value for the lake control (No. 10 sample) had a relatively small variation and thus a narrow confidence limit. If this confidence is used as the reference and the confidence limits for the other samples are similarly computed then it can be seen that if a difference between samples is significant the confidence limits of the test materials have to fall outside the confidence limits of the control, either greater or lesser. In University Lake the only samples that met this criteria of statistical significance were samples No. 7 on July 16 and No. 8 on July 8. In both cases these were additions of sodium and potassium bicarbonate. In each instance these were lower in productivity than the control.

In applying the same test criteria for the Lake Michie samples the only test condition which exceeded the confidence limits of the control was found on October 8 in sample No. 8, again a bicarbonate addition, and again producing a lower productivity. Similarly in the Lake James series on only one sampling date did one of the test media fall outside the confidence limits of the control and again it was sample No. 8 the addition of potassium bicarbonate and again the value was low.

Since productivity of a body of water as determined by the rate or amount of carbon fixed per unit time is both a function of the number of phytoplankton utilizing CO_2 and the amount of solar energy available, the relationship of these variables to carbon fixed was examined in the lake controls for the entire series. As shown in Figure 4, a tri-dimensional plot of the three variables, there appears to be a peak of productivity achieved at cell concentrations of about 2000 cells/ml and an energy input to the water surface of around 30 – 35 cal/cm^2-hr^{-1}. Higher cell numbers and higher energy inputs did not seem to produce higher carbon fixation rates and in fact as cell number increased, rate of carbon fixation actually appeared to drop. The implication of this observation may be that CO_2 is limiting under these circumstances. However, those control samples which appear to be past the peak relationship of cell number and insolation do not show enhanced productivity in those lake samples spiked with either $NaHCO_3$ or $KHCO_3$. The anomalous plankton sample from University Lake of September 30 is not included in this plot.

Batch Assay Evaluation

Batch assay cultures were carried out according to PAAP procedures. Samples for cell count determinations were taken at the end of about 1 week, 10 days, 14 and 21 days. At the end of the growth period of 21 days, dry weight determinations were made of the biomass formed. Carbon determinations were also made at 21 days, using a total carbon analyzer. Determinations of algal carbon were computed from the total carbon in the cell suspension minus the total carbon in the medium, the cells being removed by centrifugation. Thus, the effect of the various additions (Table 1) to the filtered lake waters can be analyzed with reference to the manner in which they influenced growth rate as well as final production of cellular material and cellular constituents. Figures 5-8 illustrate the cell counts plotted as growth curves for the University Lake series; Figures 9-12 show the growth response for the Lake Michie series and Figures 13-16 the growth response to the various additions to Lake Jones water. In review of these figures, it is quite apparent that a consistent pattern of response to the various added materials is seen in all the lake waters, although for each set of samples the rates of growth as well as individual fluctuations for the particular added material showed considerable variation. In an attempt to analyze these growth responses which are apparently subject to many individual variations both in changes in quality of the seeded water as well as the growth conditions for each set of samples, a set of tables were prepared which attempts to establish a pattern of frequency of occurrence of the particular response parameter in rank order. Tables 7 and 8 rank the cell count at 14 and 21 days respectively for each medium while Table 9 presents dry weight and Table 10 carbon content of the cell mass formed. Thus the pattern of rank order of response to any added substance is compared to the NAAM control medium as well as the lake water control for the five the four test dates on each lake.

In Tables 7 and 8, the initial inoculation being made at 10^3 cells, the first rank is 10^4 and on up to 10^7 cells/ml. It is seen that the maximum growth was obtained with the NAAM control medium (No. 10) and the two sewage samples, media No. 2 and No. 4, No. 2 containing NTA whereas 4 is the secondary effluent without the NTA. Tertiary effluents No. 3, with NTA from a secondary treated source, and No. 5, a tertiary effluent without NTA, appear in a consistent pattern at the next lower rank level of 10^6 cells. In only two lake samples, both from Lake Jones on August 27 and September 24, does the No. 1 sample, containing 4 mg/l of NTA, produce cell growth at the 10^6 level. In Lake Michie and University Lake water as well as in Lake Jones, a more consistent pattern is for the No. 1 sample to show a growth response that places it in the rank order of cell number of 10^4 or 10^5 cells after 14 or 21 days incubation.

The rank order response, as measured by dry weight determinations, shows a similar distribution although with somewhat greater scatter. The NAAM medium

Table 7

NTA Evaluation

Rank Order Comparisons of Cell Growth (Cell No./ml) in the Various Media at 14 days Incubation

July – October, 1970

Cell No./ml	University Lake 7/16	8/13	9/9	9/30	Lake Michie 7/28	8/19	9/16	10/8	Lake Jones 8/6	8/27	9/24*	10/15
10^7												
	1 4 2	1 2 4	4 2	1 2 4	1 2	1 2 4	1 4 2	1 2 4	2,4 1	1 4 2	2,1	1 4 2
10^6												
	6 3 10 9 8	3 9	9 3	9 2 5	4 3 9	3 9	3 9	3 5 9	3 9	3 5 9 11	5 4,3 11 10 9,7 8	3 12
10^5												
	7 5 11	5,6 11	5	10 8 7 11 6	5 7 8	5	5	6 10 7 8 11	5 6 11 10	7 6 10 8	6	5 9,6 10 11 7 8
10^4												
		7 8 10	10 7,8 11 6		11 6 10	8,10 11,7 6	11 8,10 6 7		8 7			

*Measured at 17 days

Table 8

NTA Evaluation

Rank Order Comparison of Cell Growth (Cell No./ml) in the Various Media at 21 Days Incubation

July – October, 1970

Cell No./ml	University Lake 7/16	8/13	9/9	9/30	Lake Michie 7/28	8/19	9/16	10/8	Lake Jones 8/5	8/27	9/24	10/15*
10^7												
	1 4 2	1 4 2	2 4	1 4 2	1	1 2 4	1 2 4	1 4 2	1 2,4	1 4 2 3	1 2	1 2 4
10^6												
	3 9	3,9	9 3	9 3 5	3 2 9 4	3 9	3 9	5 3 9	3 9	5 9 11	4 11 5 9,10 7,8 3,6	3 5,10 7 12
10^5												
	7 10 5	5 7	5 10	10 11 7 6,8	5 7 8 6	5 10 7 6	11 10	10 6 7 11 8	10 5 6 11	6 7,10 8		9 6 11 8
10^4												
	11,8 6	11 6 8 10	11 7,8 6		10 11	8 11	5 6,7 8		8 7			

*Measured at 19 days

control as well as the No. 2 and No. 4 samples outrank in dry weight production all other media. On the basis of this criterion, the additions of NTA showed little difference in response to that of the unspiked lake control. The carbon determinations at 21 days showed a somewhat tighter ranked order but again the maximum carbon production was in the NAAM media control followed by the No. 2 and No. 4 samples, the secondary sewage effluents with and without NTA. All other samples rank much lower with the untreated lake controls, the No. 10 sample, just as frequently producing equal to or better than the other spikes in terms of carbon production. (Tables 9 and 10.)

Calculations of confidence limits for each of the lake series based upon cell count, dry weight and carbon production were also determined and are presented in Figures 17 - 28. Due to the considerably greater degree of variability between duplicate samples, the 90% confidence brackets tend to be fairly broad. However, one thing is clearly indicated - that the presence of NTA in no way produces a growth response which significantly exceeds the 90% confidence limit of the lake control.

Another comparison that might be made of the response of algal growth in the batch assay to various additives is shown in Figures 29 - 34. For each of the four samples from the three lakes, a histogram is presented for each medium comparing the log cell number with the total calculated quantity of nitrogen and phosphorus present in that particular medium and the particular lake water of that date.* The NAAM control sample, No. 11 and the lake water of that date provide a comparison to the other media. Media No. 1, 2, and 3 are those in which NTA is present. Media No. 4 and 5 are the sewage samples which contain no NTA. It is of interest to note the University Lake series, Figures 29 and 30, the medium which had the largest cell growth after the sewage secondary effluent samples (2 and 4) was No. 9 containing .01 mg/l P. This amount of phosphorus was added in smaller quantity than originally planned, which was to be .05 mg/l. One interesting

Table 9

NTA Evaluation

Rank Order Comparison of Cell Growth (Dry Wt. mg/l) in the Various Media (Numbers 1-12) at 21 days Incubation

July – October, 1970

Dry Wt. (mg/l)	University Lake 7/16	8/13	9/9	9/30	Lake Michie 7/28	8/19	9/16	10/8	Lake James 8/6	8/27	9/24	10/15
>1000		11		11	11		11	11		11		11
751-1000	11										11	
551-750				2		11						
401-550		2		4				2 4	11 4 2			
301-400	4 2	4	4		4 2		4 2			4 2	2	
201-300			2			4 2					4	4
101-200		3		9	3 9			3	3	3	3	2 3
1-100	3 9 7 5 10	9 5 6 1 10 7	3 9	3 5 8 10 6 7 1	5 6 1 8 10 7	3 5	3 9 8 5 10	5 6 9 1 7 8,10	9 5 6 10 1 7	5 9	5 6 7 9 10 8 1	5 6 12
<1.0	6 1 8	8	5 10				6 1 7		8	1 6,10 7, 8		9 1

Table 10

NTA Evaluation

Rank Order Comparison of Cell Growth (Carbon - mg/l)
in the Various Media (Numbers 1-12) at 21 Days Incubation

Carbon (mg/l)	University Lake 7/16	8/13	9/9*	9/30	Lake Michie 7/28	8/19	9/16	10/8	Lake Jones 8/6	8/27	9/24	10/15
725-1000							11	11				11
500-724	11	11		11	11					11		
401-500						11					11	
301-400				2 4					11			
201-300	4	2	4 2		2		2	2 4	4 2	2	2	2 4
101-200	2	4			4	2	4			4	4	3
1-100	3 9 8 7	9 3 5 7 6	3 9 7 6 10 5	9 3 8 5 10 6	3 9 5 10 8 6 7	4 3 5	3 9 5,10 8	3 5 8 6 1,10	3 [illegible] 6 5	3 9 5 1 7	5 3 6 1 9 10	5 9 6 12 7 1,8
<1.0	1,6 5,10	10 1,8	8 1	7 1	1	9 7 1,6,8,10	1,6,7	9 7	10 7 1,8	6 10 8	8 7	10

*No Number 11 Medium

comparison that can be made from these figures in the examination of the relative growth response in the media 2 and 4 as compared to 3 and 5. The presence of NTA in the secondary effluent appeared to make no difference in the growth response. In several instances, the presence of NTA in the tertiary effluent in all the University Lake samples and those of the Lake Michie samples appeared to induce a slightly greater growth response. However, in the Lake Jones samples the presence or absence of NTA in a tertiary effluent is somewhat more variable in one instance the growth response in the media containing the tertiary effluent without NTA being greater than with NTA.

Still another measure of growth behavior in these various media prepared with lake waters is the measurement of pH after various periods of incubation. This response is shown in Figures 25-28. The pattern of response reflects the relative nutrient richness of the media associated with rapid growth in the waters containing sewage effluents as well as in the NAAM media. This is consistent throughout the test series except the samples from Lake Michie on September 16 and Lake Jones of September 24 which showed no high pH value developing in the sewage media. However, in the same series the NAAM control showed the usual high pH values at 6 and 11 days of incubation.

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* All amounts of N and P recorded are based on measurements made in the U. N. C. laboratories.

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CONCLUSIONS

1. Free NTA added to three lake waters on four different occasions showed no significant change in rate of carbon fixation (carbon-14 test) or in batch assay as measured by cell number, cell mass, or carbon production.

2. The presence of NTA in secondary or tertiary sewage effluents showed no significant difference in carbon-14 measurements of carbon fixation rate or other growth response parameters by batch assay.

3. Nitrogen and carbon additions to lake waters in amounts equivalent to that found in NTA were also used to spike lake waters but in no instance were the growth responses significantly greater than the lake control. In most cases there was no significant difference between the growth produced by the equivalent nitrogen addition and NTA itself.

4. Even though a phosphorus supplement was added inadvertently at a concentration 1/5 specified, it still produced in nearly every case growth greater than the control, the nitrogen supplement or the NTA addition.

5. The supplements of $CO_3^{=}$ appeared to either produce a slightly lower growth response as compared to controls or no better than equal to the controls.

ACKNOWLEDGEMENTS

The results of this investigation reflect not only the efforts of the individuals noted on the cover sheet who made possible completion of a considerable task in a relatively short time but also the wholehearted cooperation of other individuals and organizations. The access to and use of facilities of Lake James was made possible by the full cooperation of the Duke Power Company through Mr. Charles Davey, Jr., Principal Environmental Engineer, Mr. George T. Swearingen, Manager, Environmental Health Department and Mr. and Mrs. Ralph Wallace of the Mill Power Club House on Lake James. Access to Lake Michie was through the courtesy of the Department of Water Resources, City of Durham, Mr. Wade Brown, Director.

Mr. Ben Breedlove and Mr. Martin Gantz, graduate students, did the plankton and hardness determinations respectively. Mr. Tony Evans, Research Analyst II of the Department, produced the N and P analyses from the autoanalyzers. Mr. Tony Anderson, graduate student, assisted in the field determinations.

REFERENCE

1. Dickman, M. "A New Method for Making Permanent Phytoplankton Mounts," Hydrobiologia, 31: 161-167 (1968).

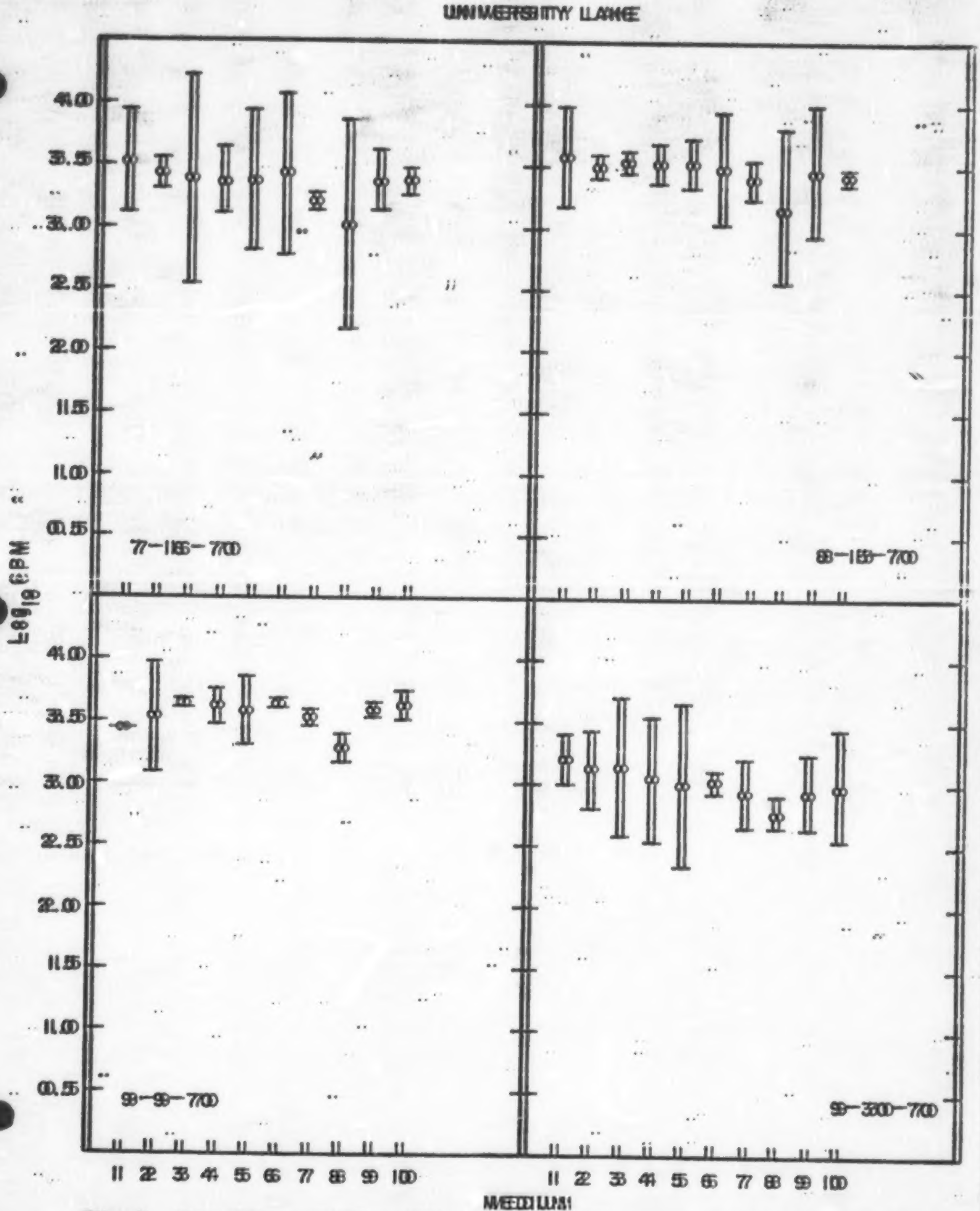

Fig. 1. Mean value and 90% confidence limits, C-14 counts, In Situ Productivity Experiments, NTA Evaluation.

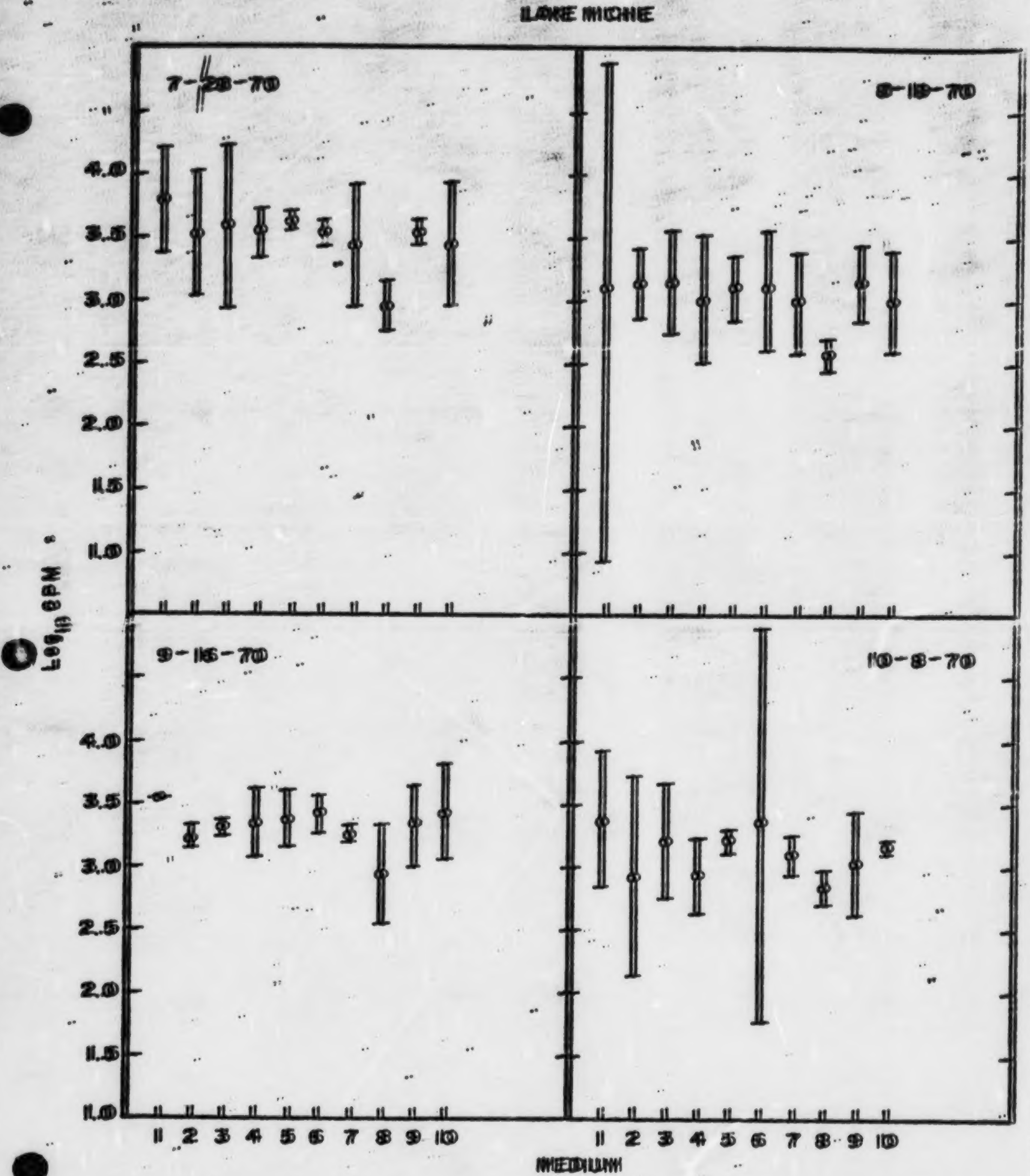

Fig. 2. Mean value and 90% confidence limits, C-14 counts, *In Situ* Productivity Experiments, NTA Evaluation.

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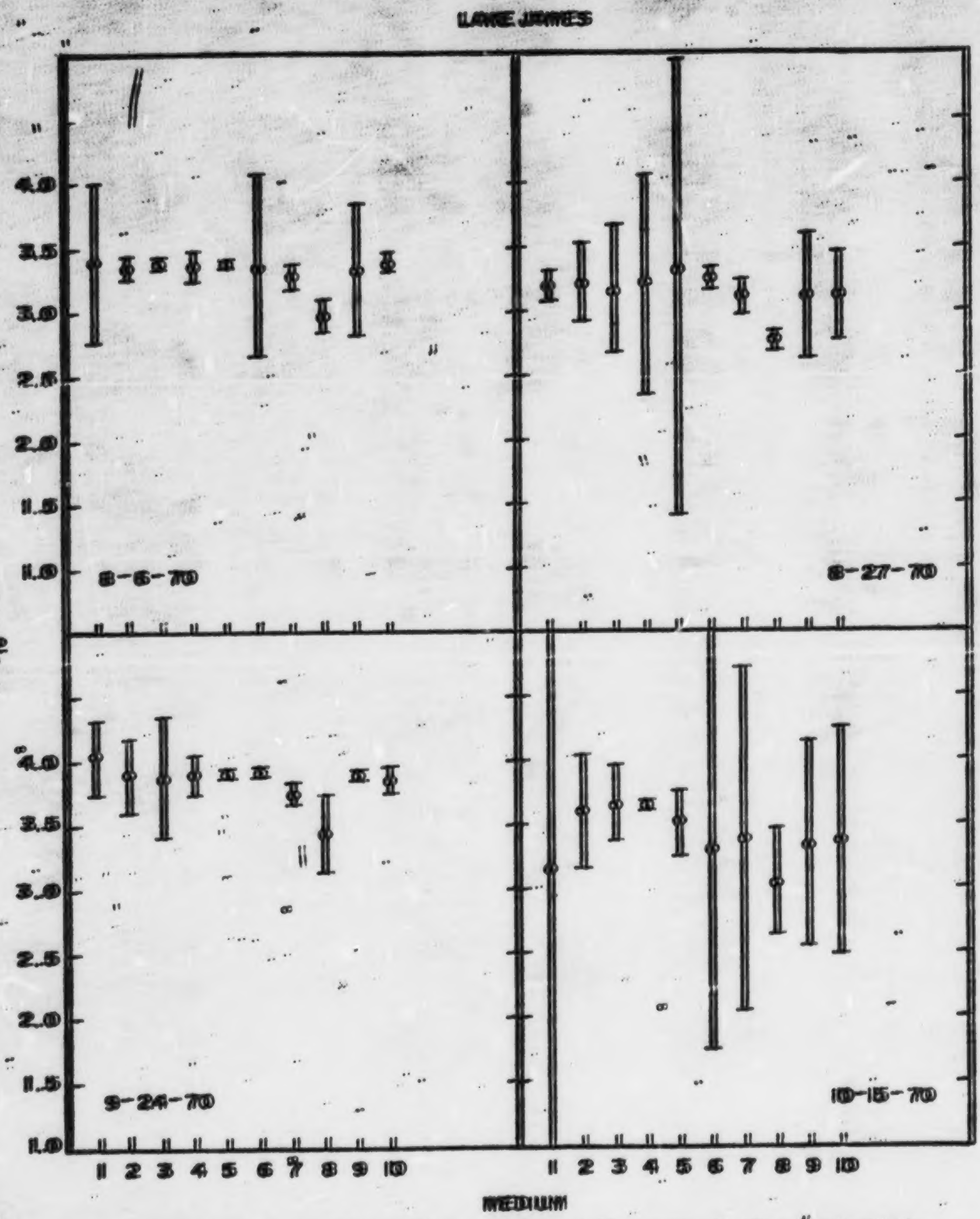
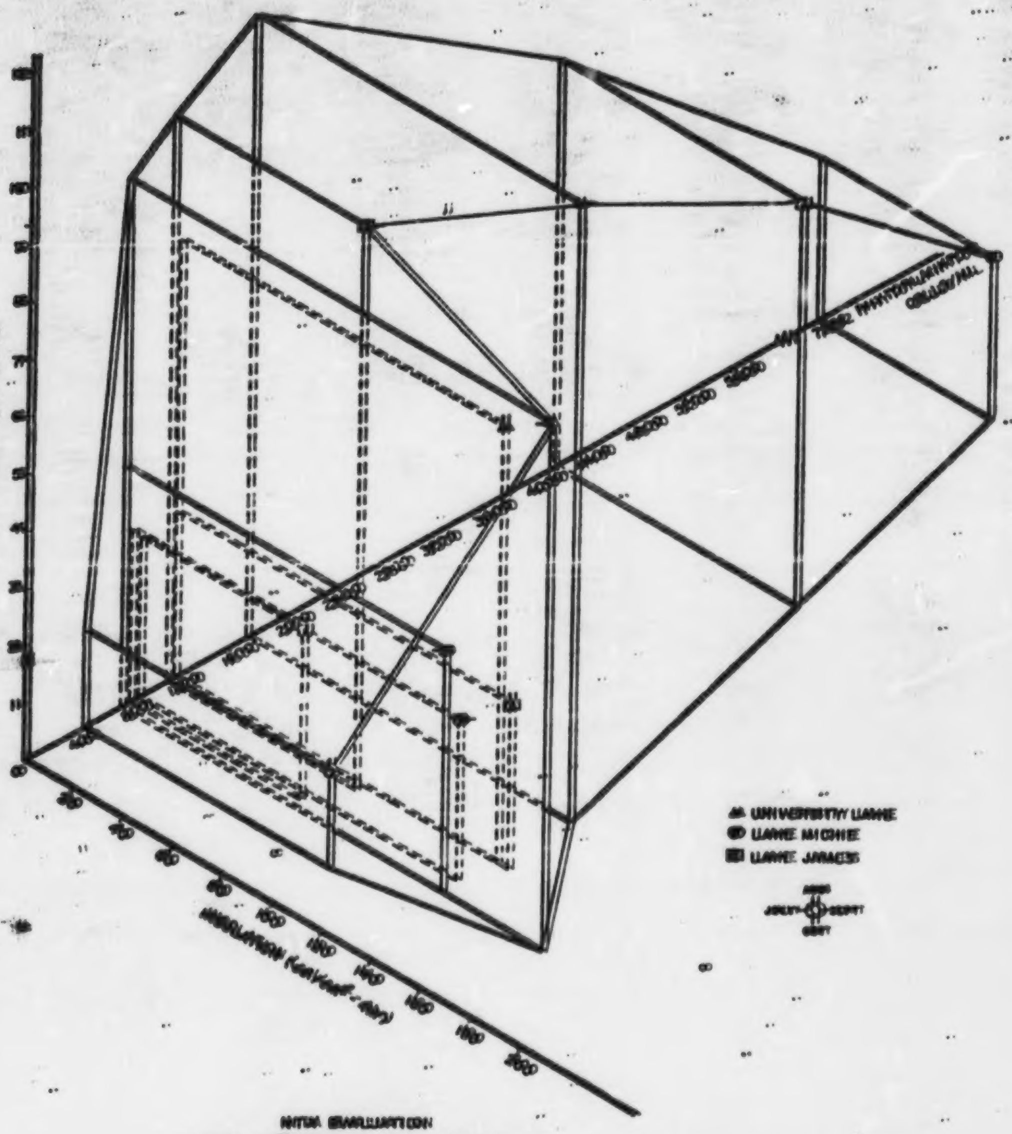


Fig. 3. Mean value and 90% confidence limits, C-14 counts, In Situ Productivity Experiments, NTA Evaluation.



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LAKE CONTROLS JULY - OCT 1970

Fig. 4. Relationship of productivity as carbon fixed to total phytoplankton numbers and solar insolation. The Sept. 30 University Lake sample is not included in this figure and the Aug. 6 Lake James sample is designated the July sample to simplify identification.

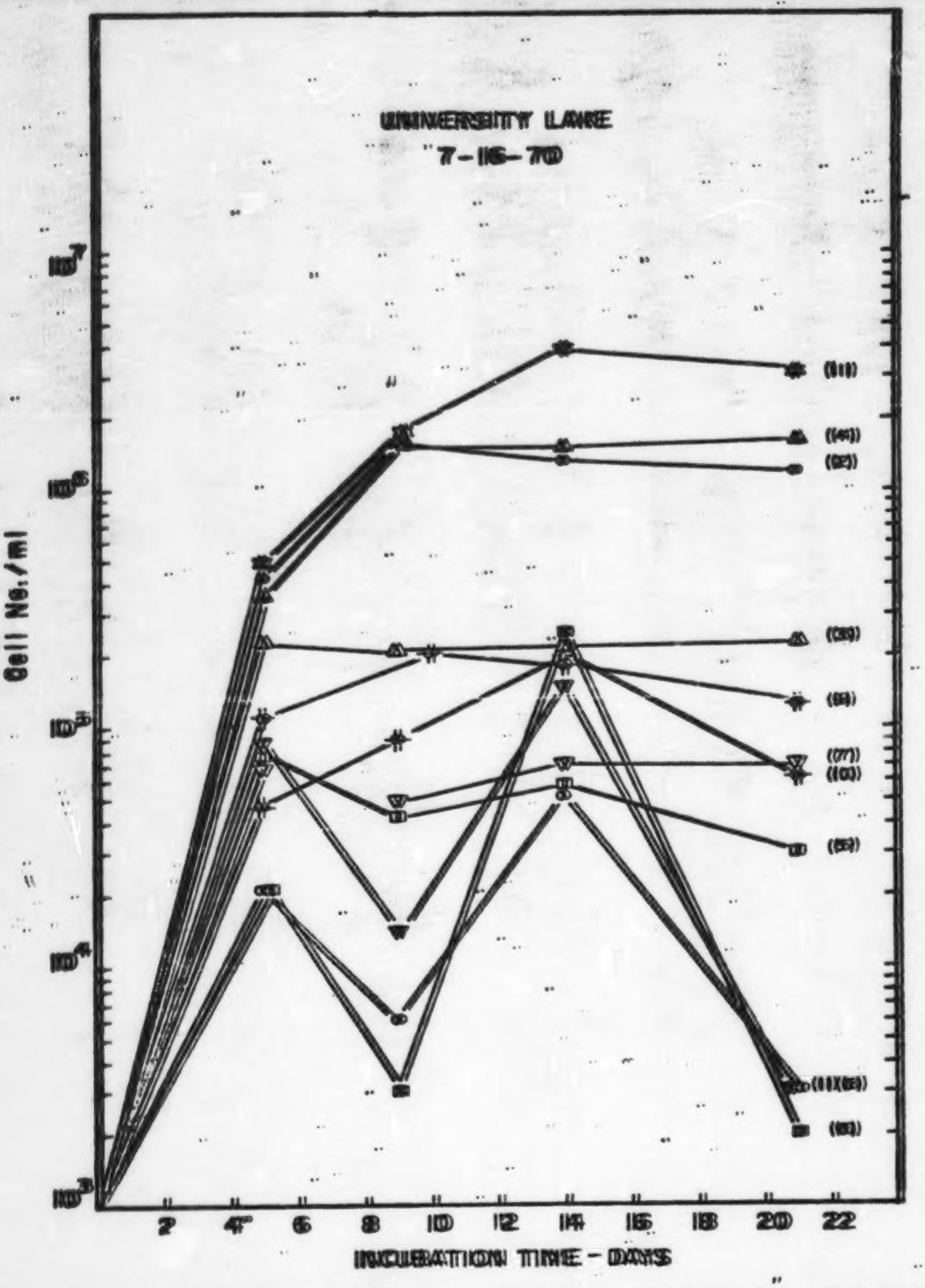

Fig. 5. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

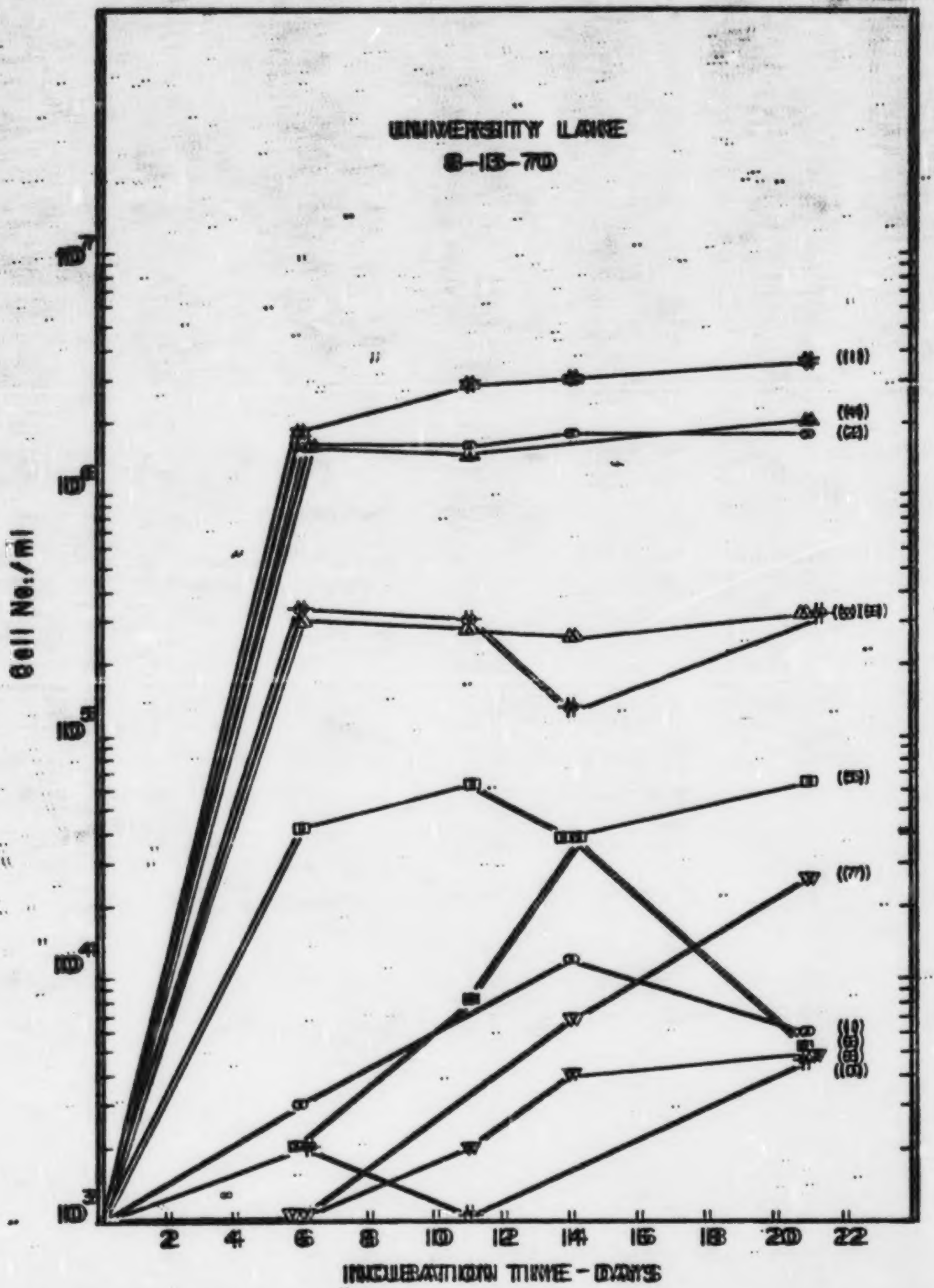

Fig. 6. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

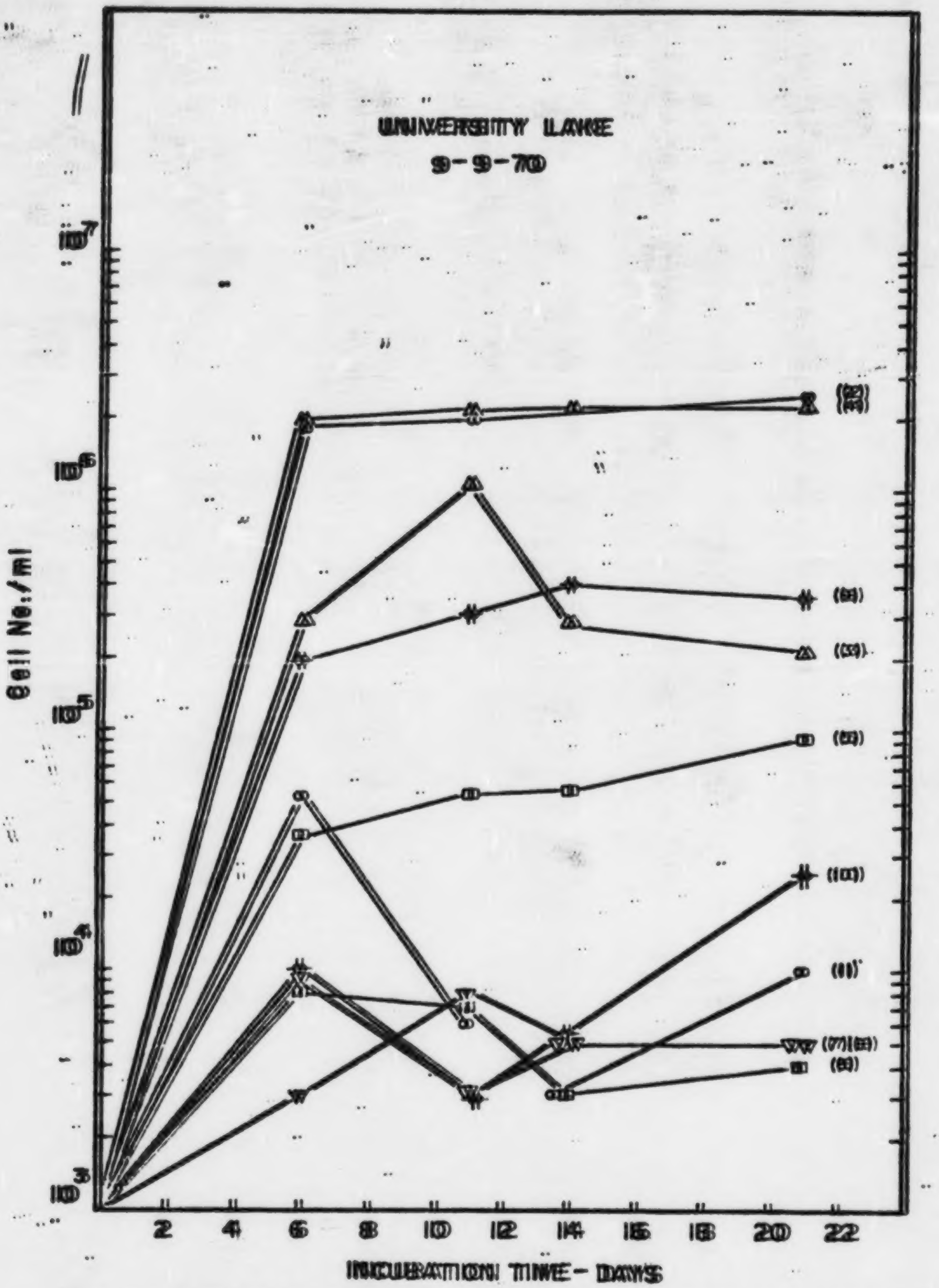

Fig. 7. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

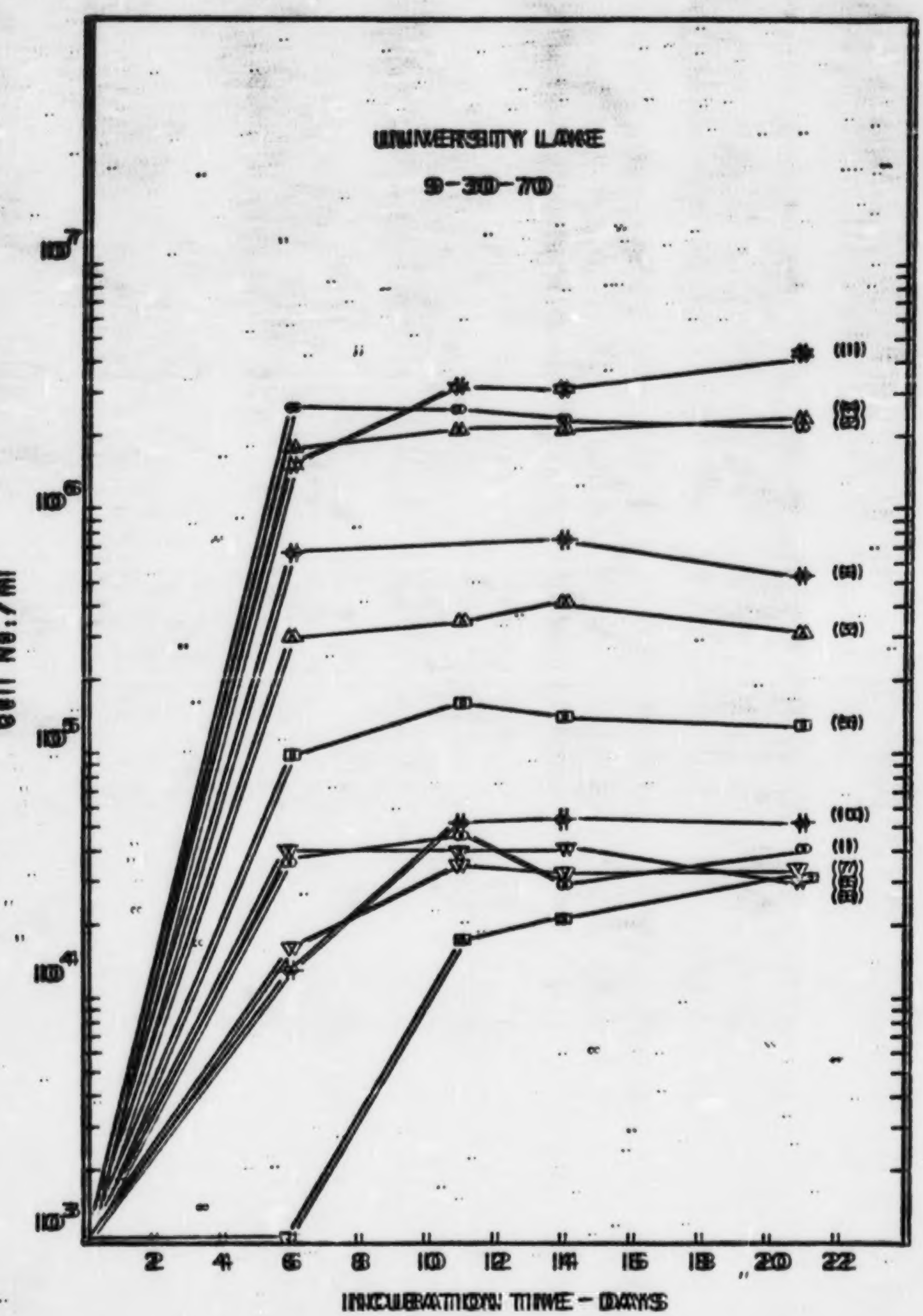

Fig. 8. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

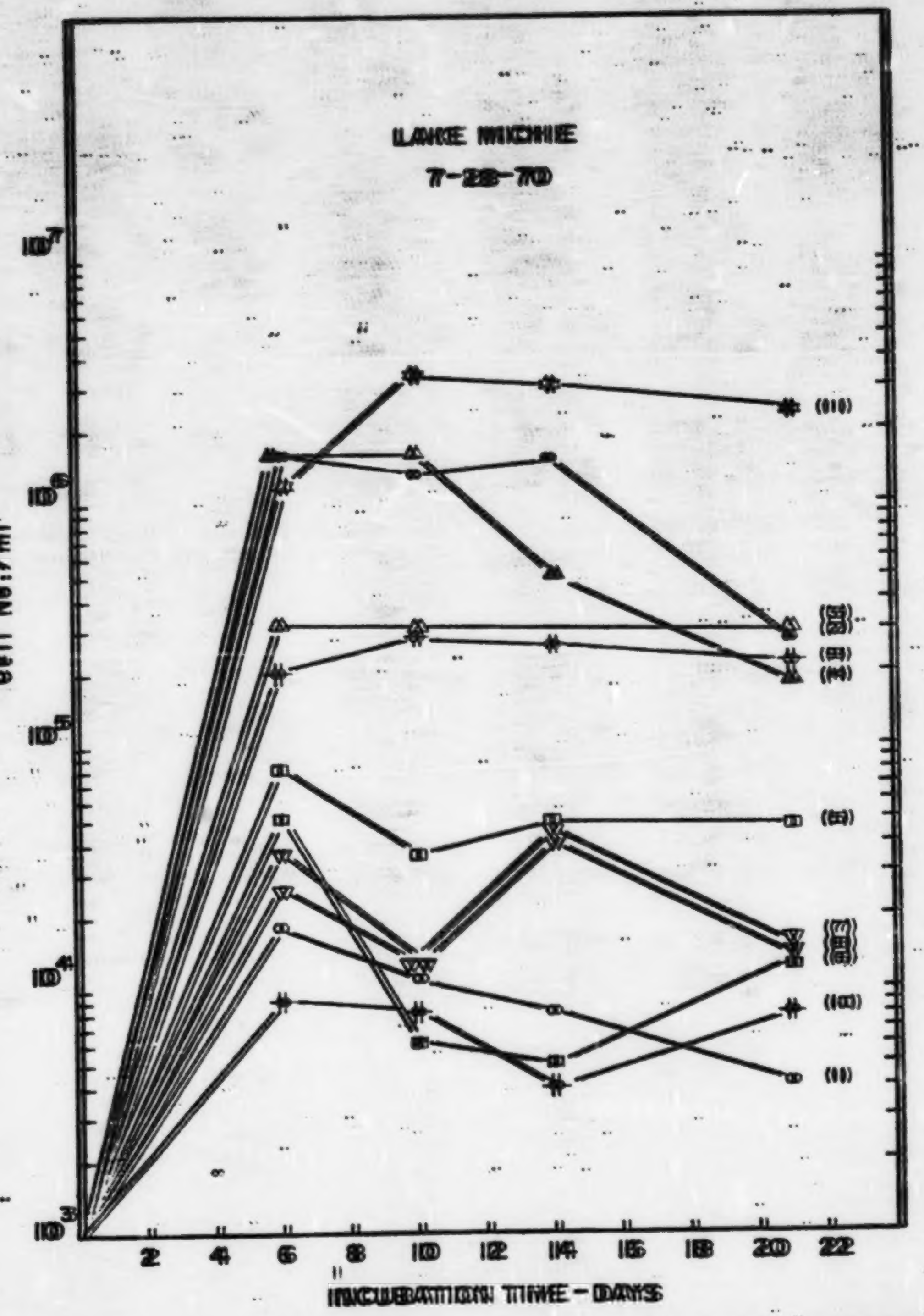

Fig. 9. Growth of *S. capricornutum* -- Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

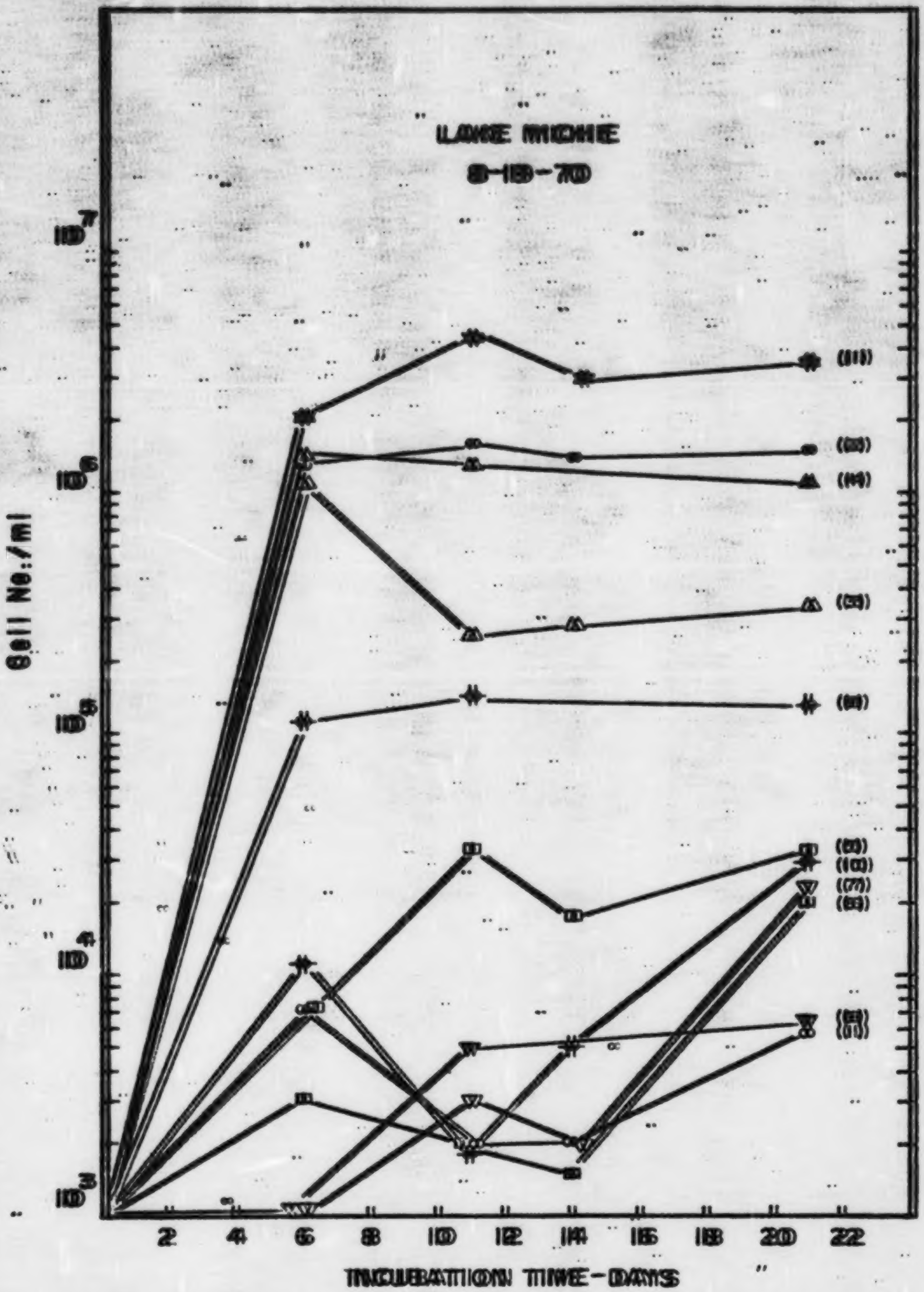

Fig. 10. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

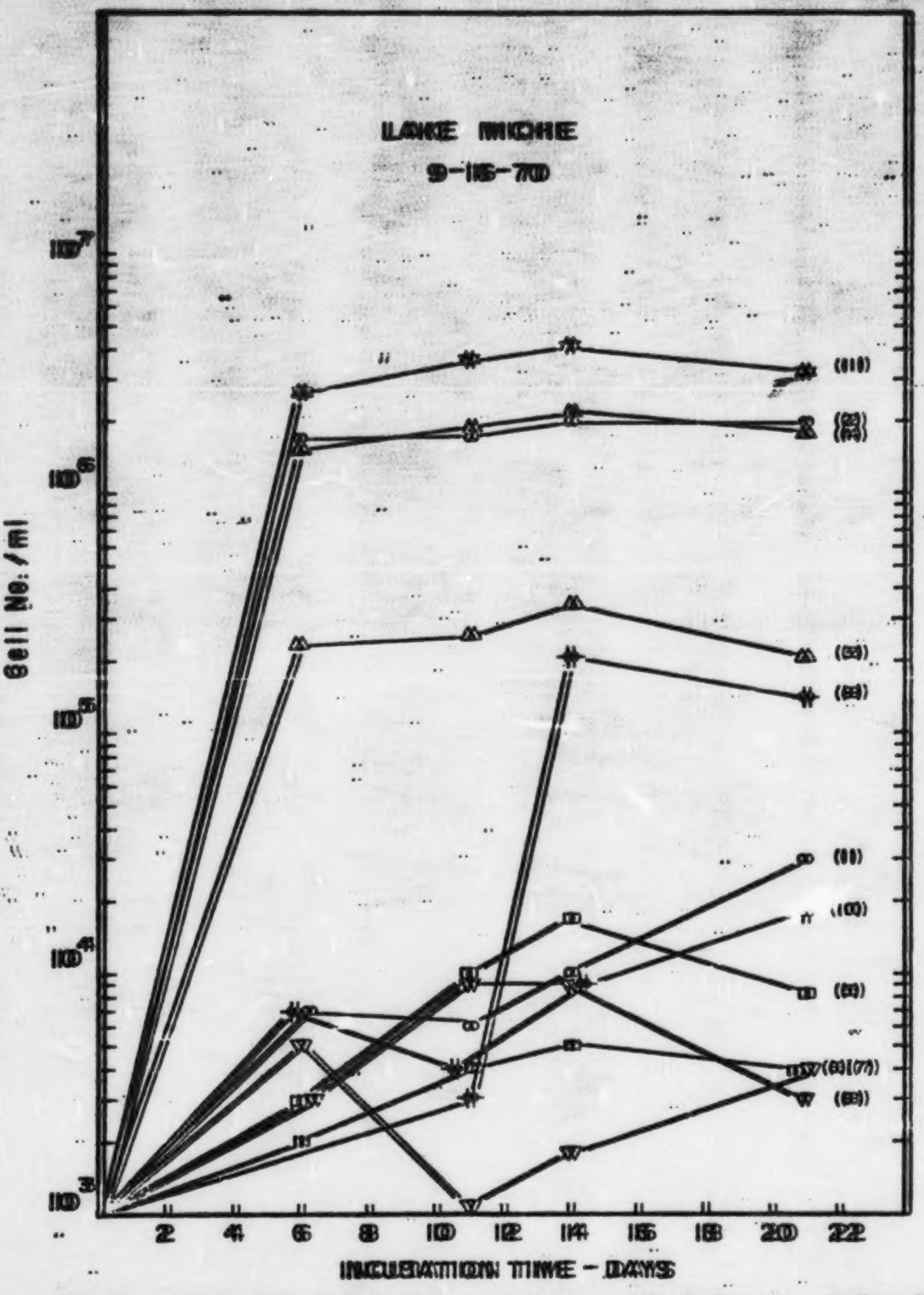

Fig. 11. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

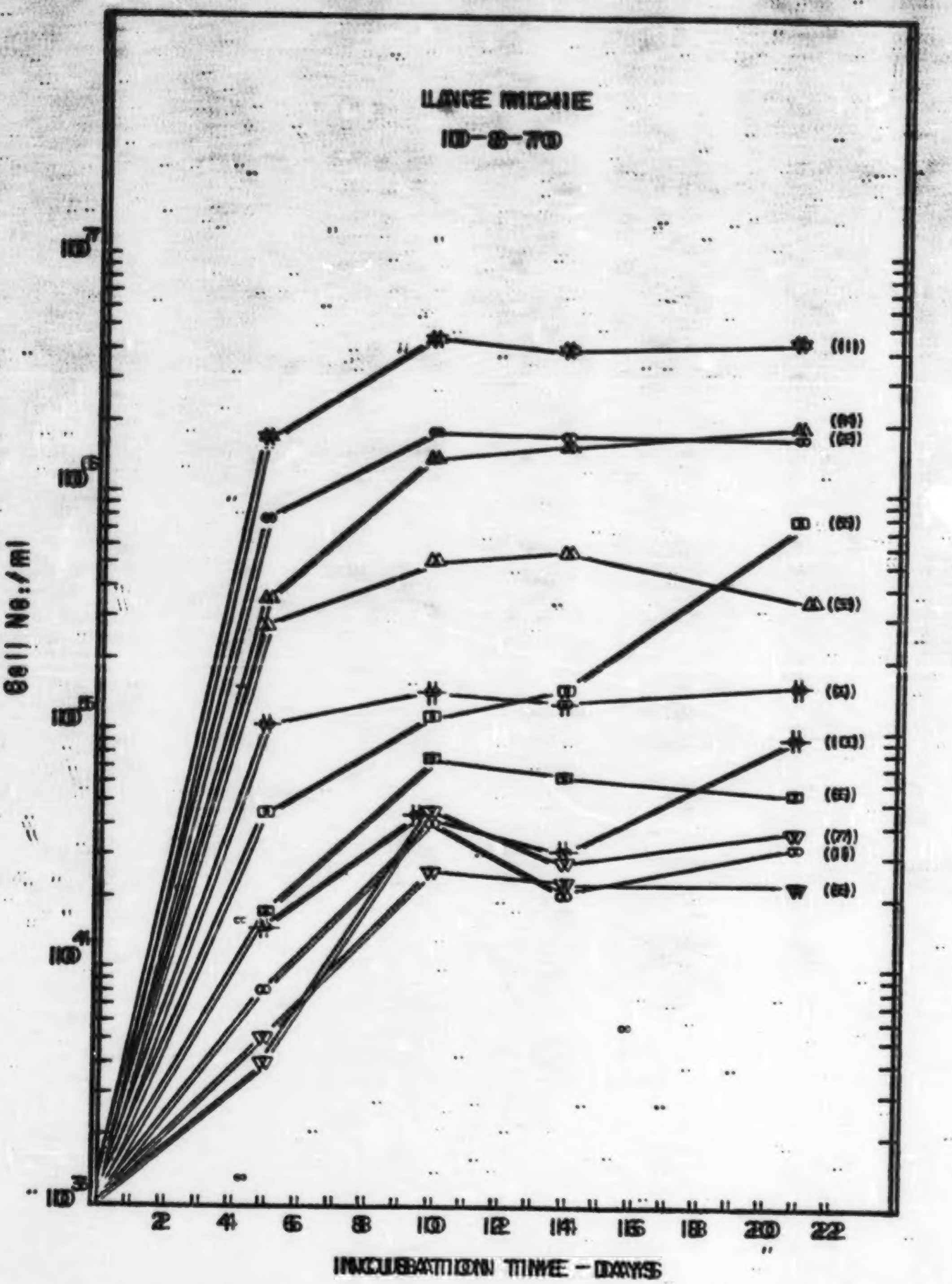

Fig. 12. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

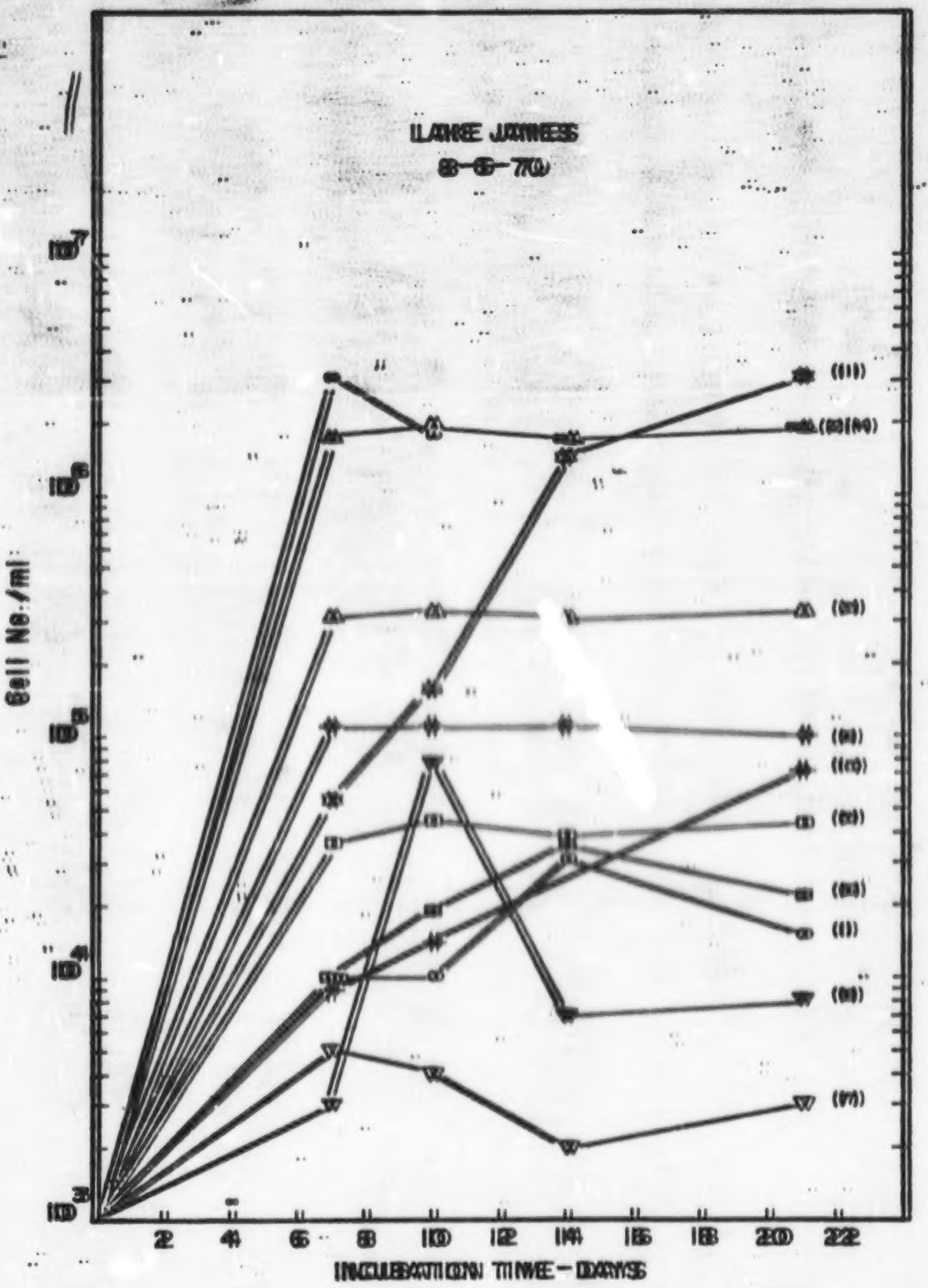

Fig. 11. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

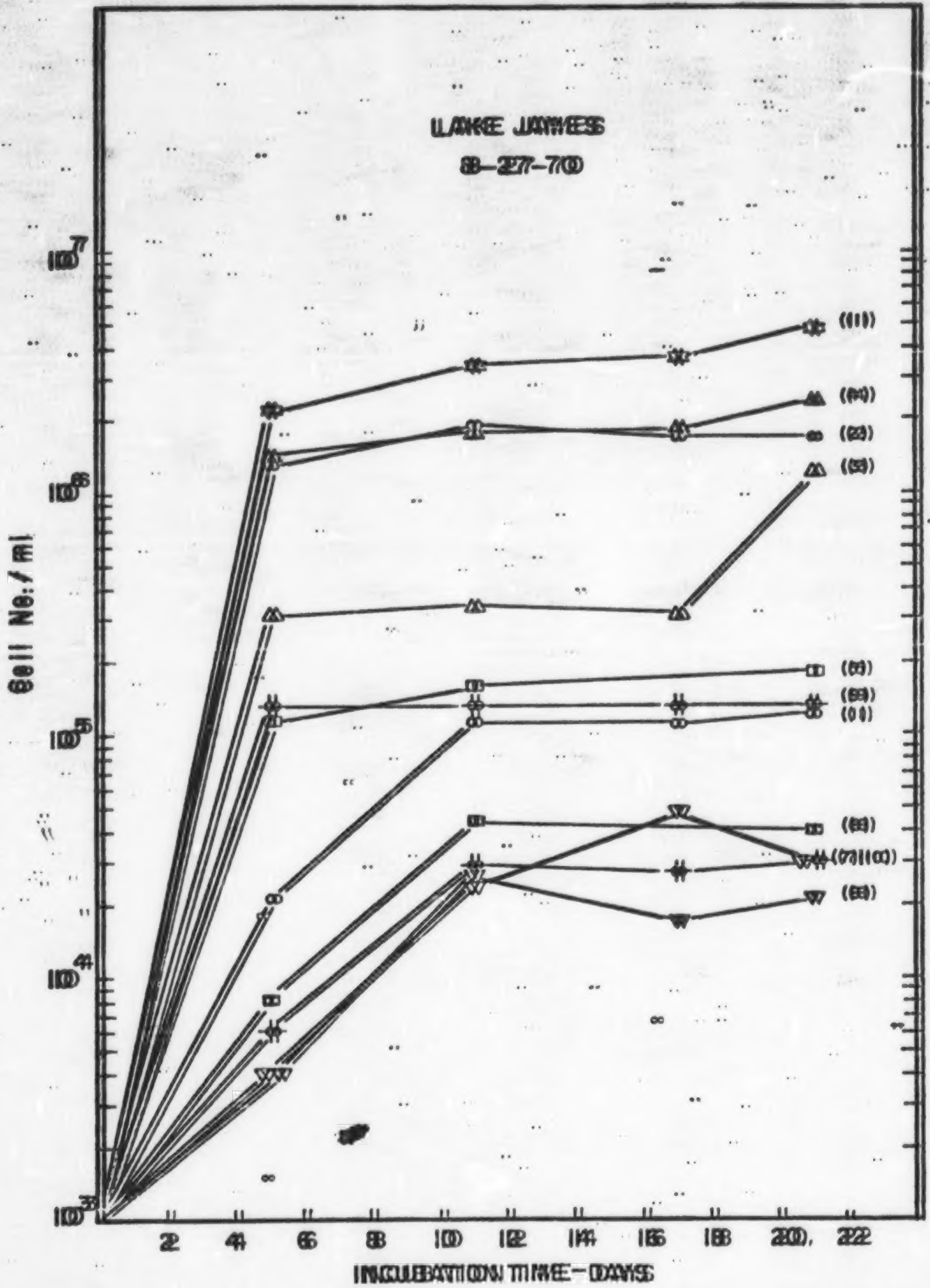

Fig. 14. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

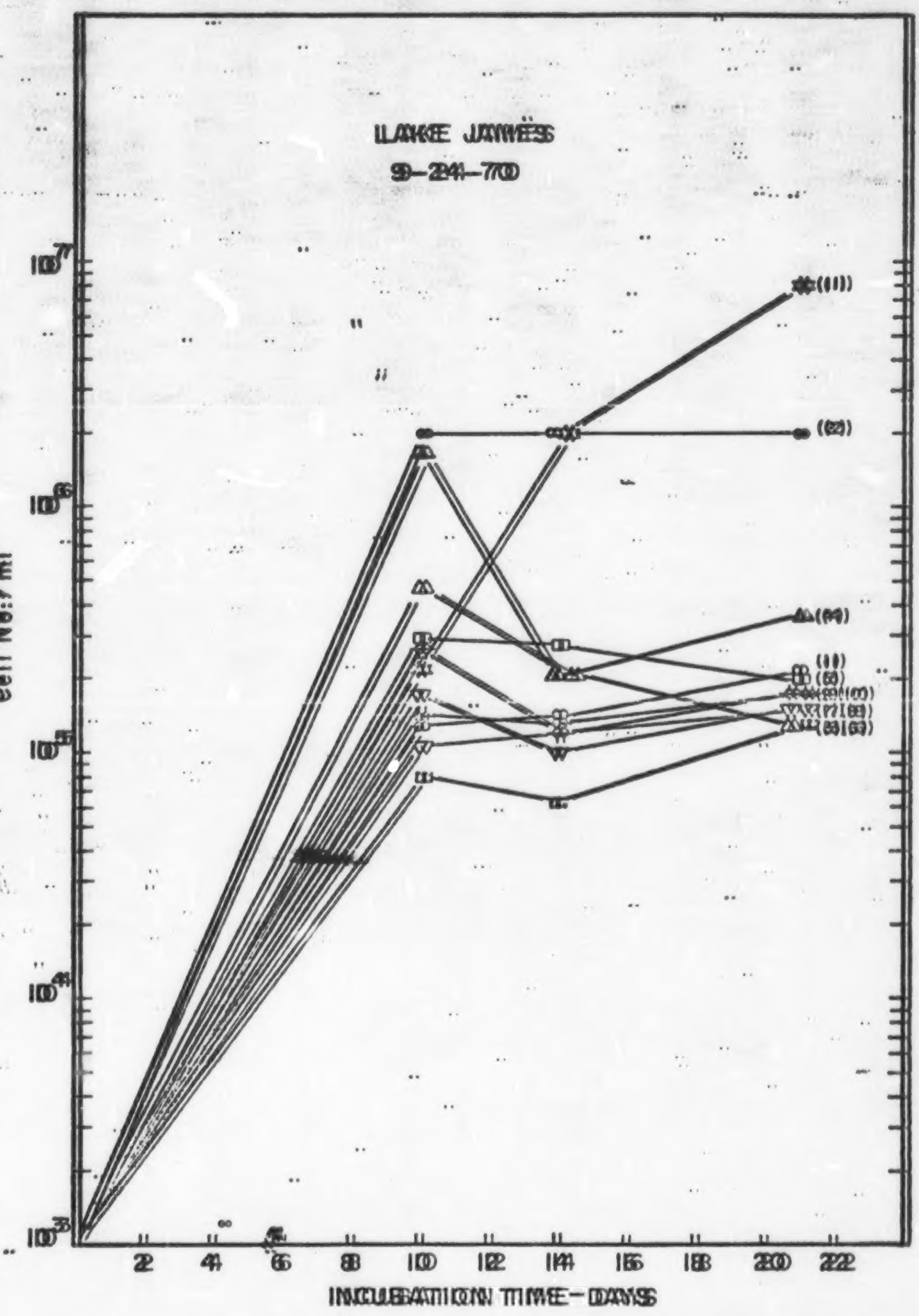

Fig. 15. Growth of S. capricornutum – Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

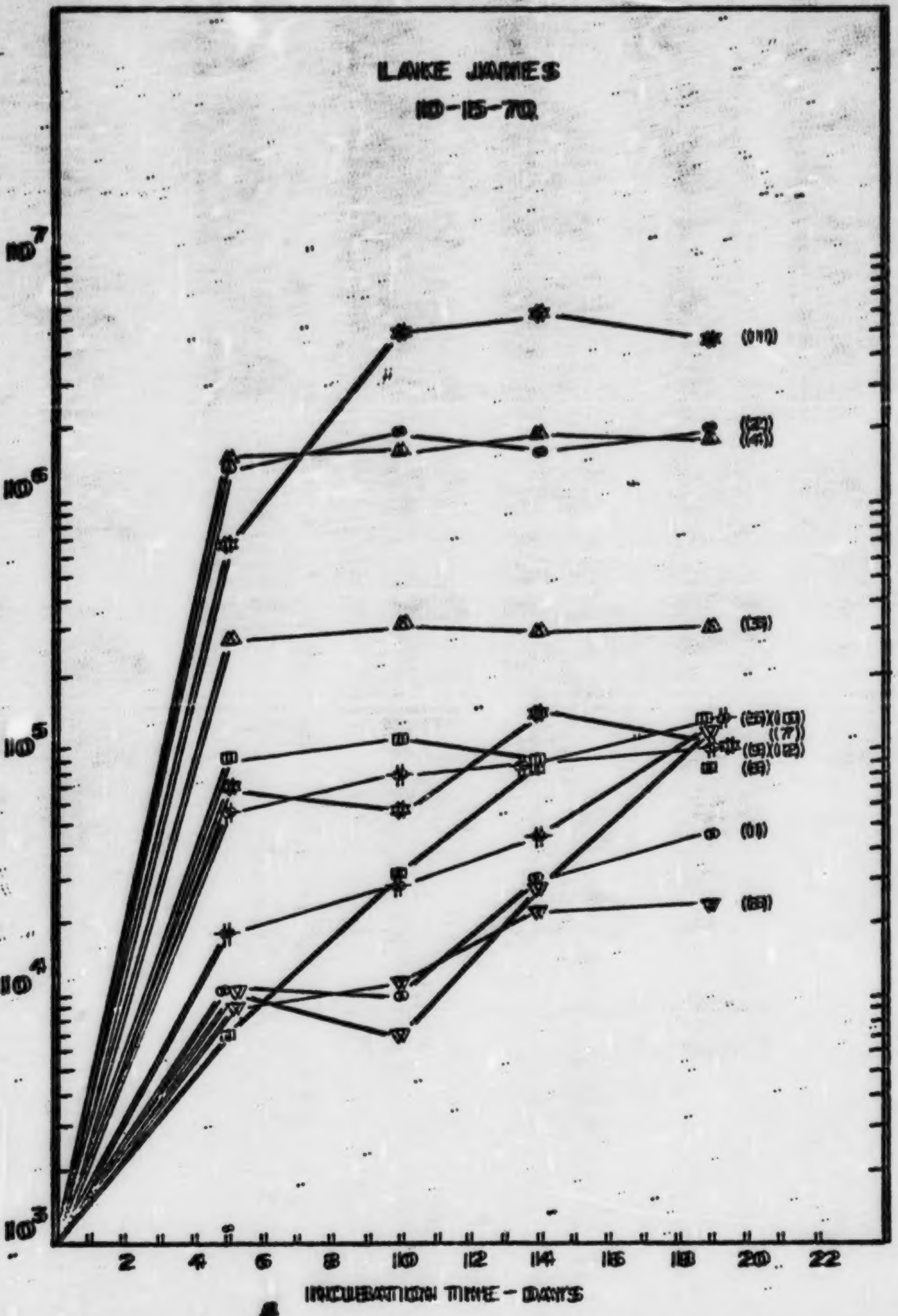

Fig. 16. Growth of S. capricornutum - Batch Assay, NTA Evaluation. Numbers in parentheses refer to media, Table 1.

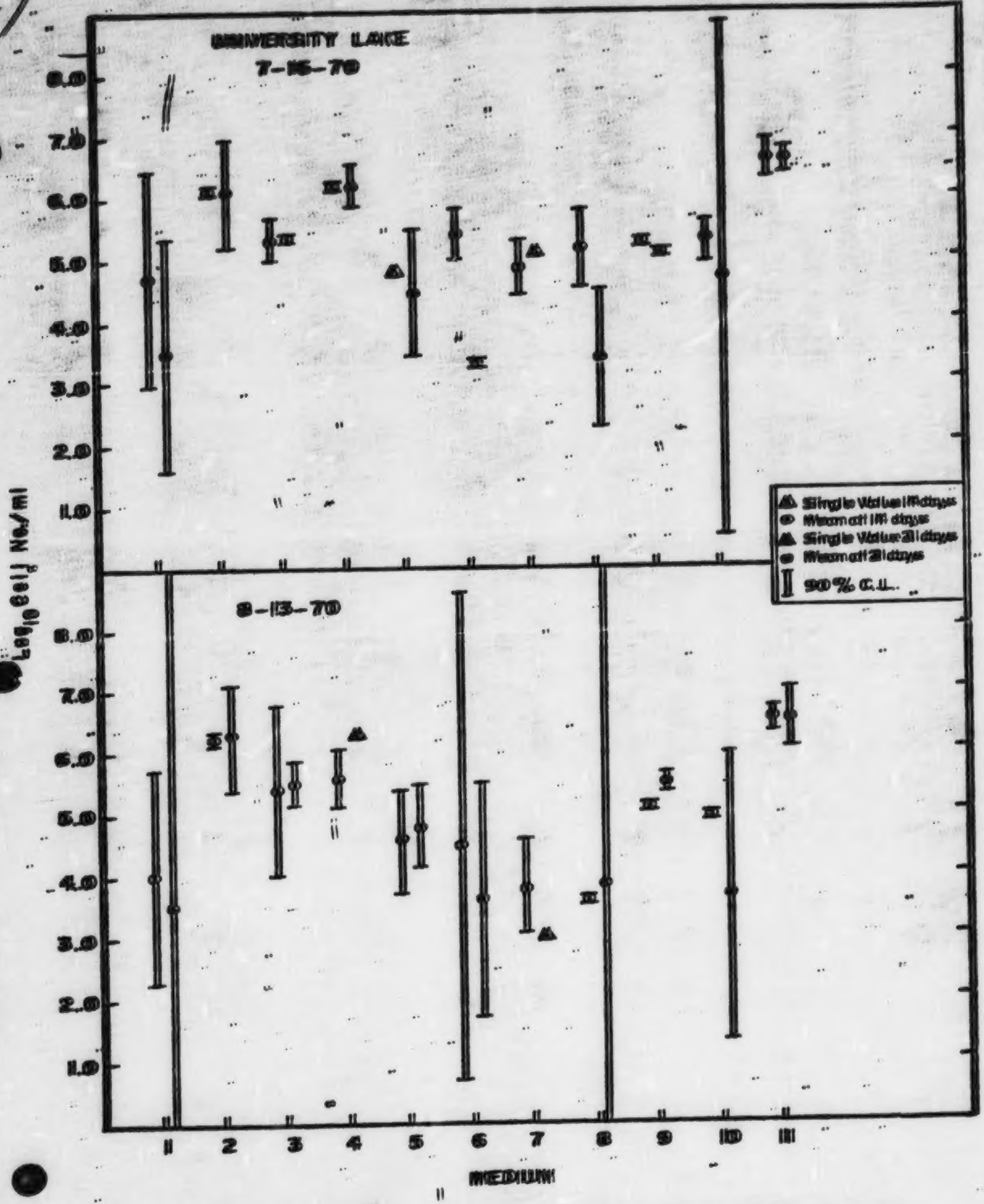

Fig. 17. Log cell number/ml, mean values and 90% confidence limits at 14 and 21 days - Batch Assay, NTA Evaluation.

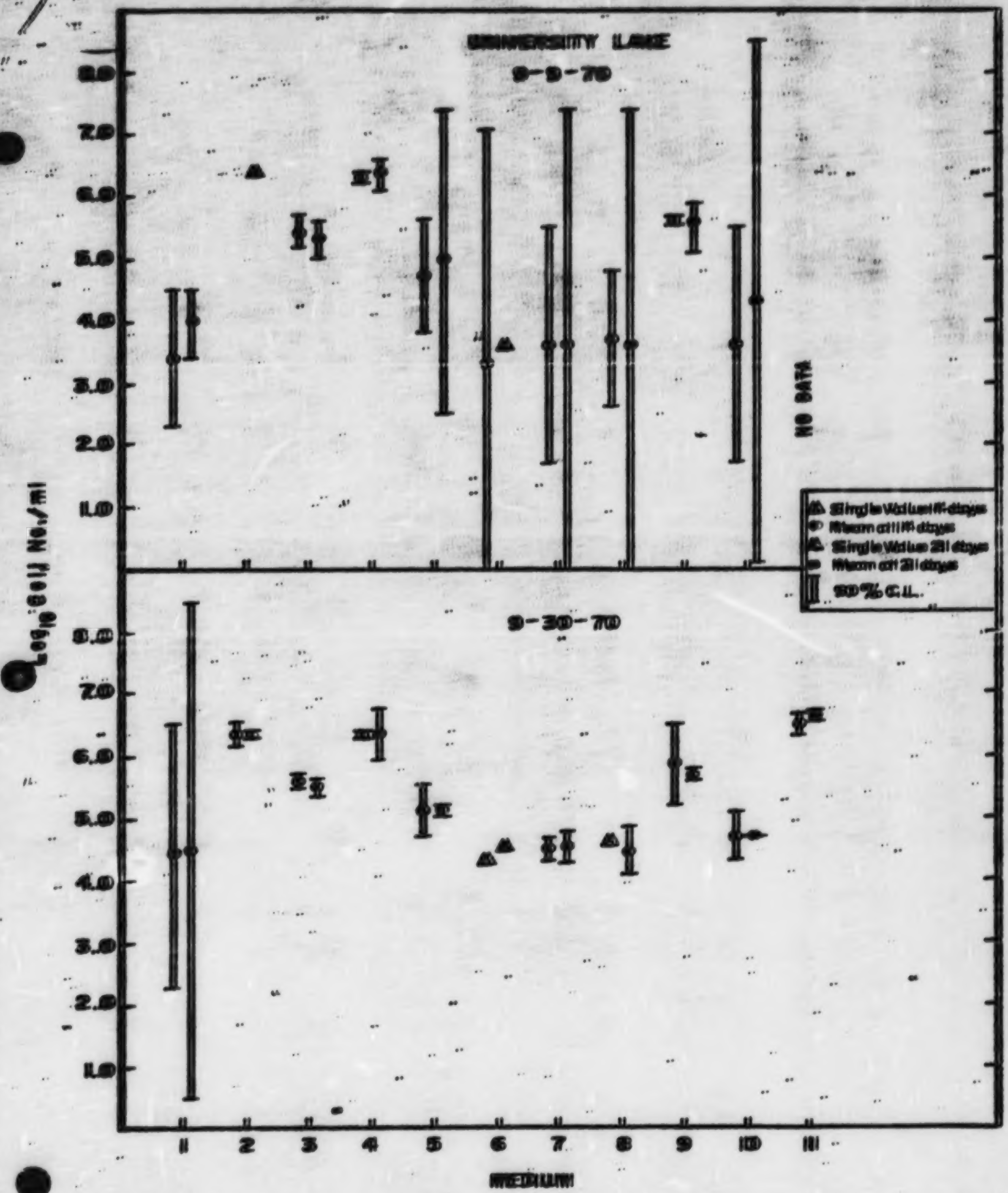

Fig. 18. Log cell number/ml, mean values and 90% confidence limits at 14 and 21 days – Batch Assay, NTA Evaluation.

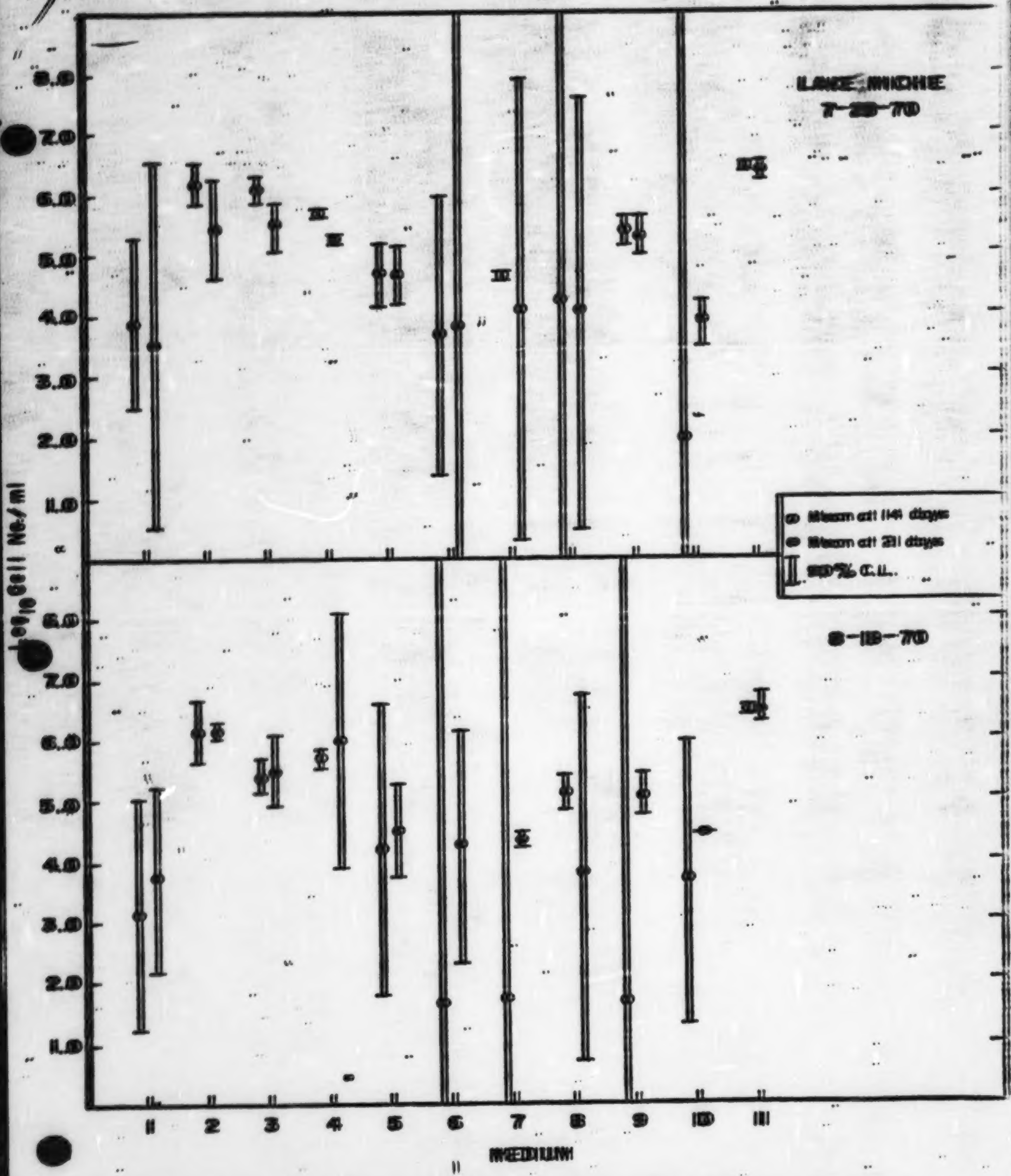

Fig. 19. Log cell number/ml, mean values and 90% confidence limits at 14 and 21 days — Batch Assay, NTA Evaluation.

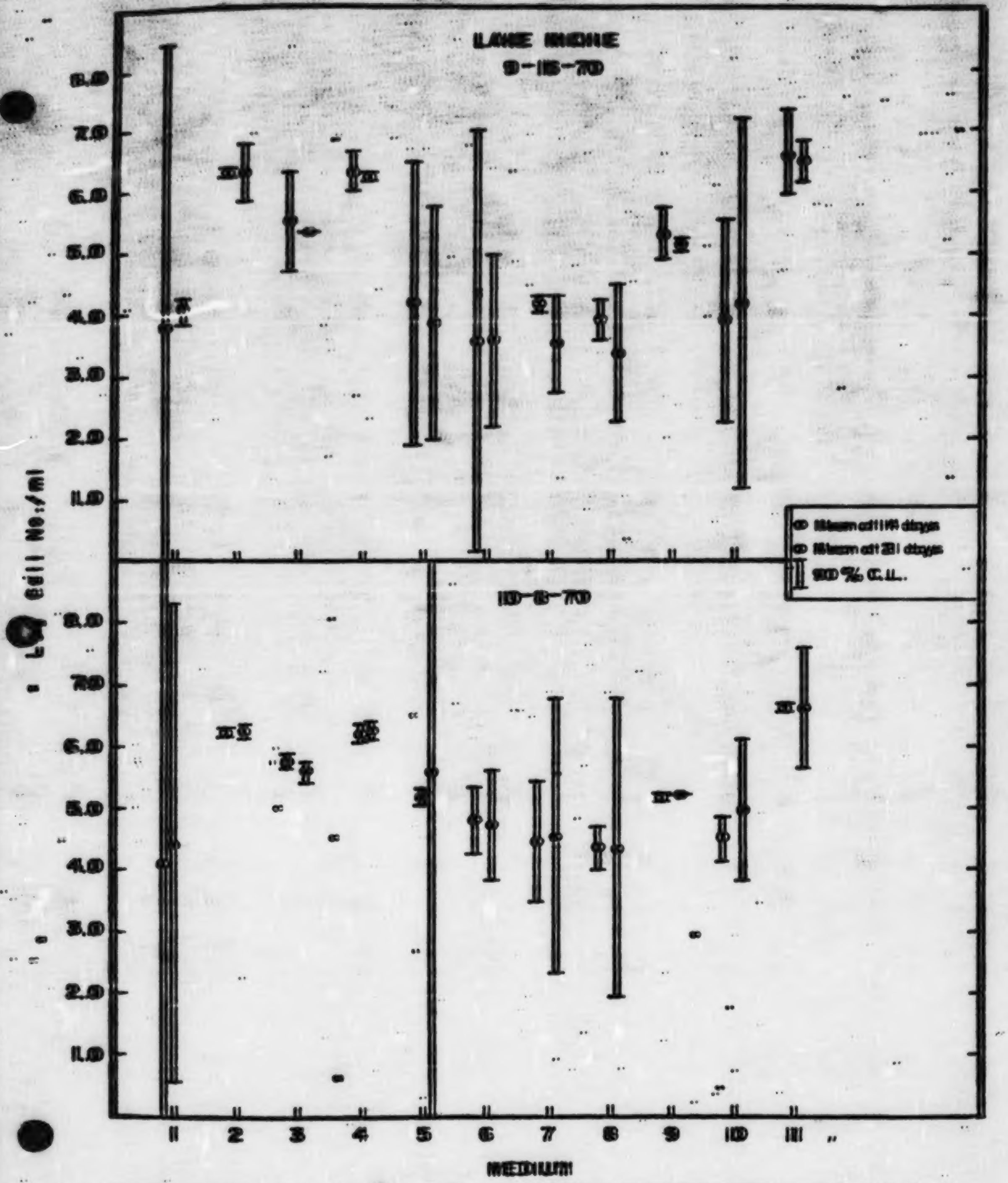

Fig. 20. Log cell number/ml, mean values and 90% confidence limits at 14 and 21 days - Batch Assay, NTA Evaluation.

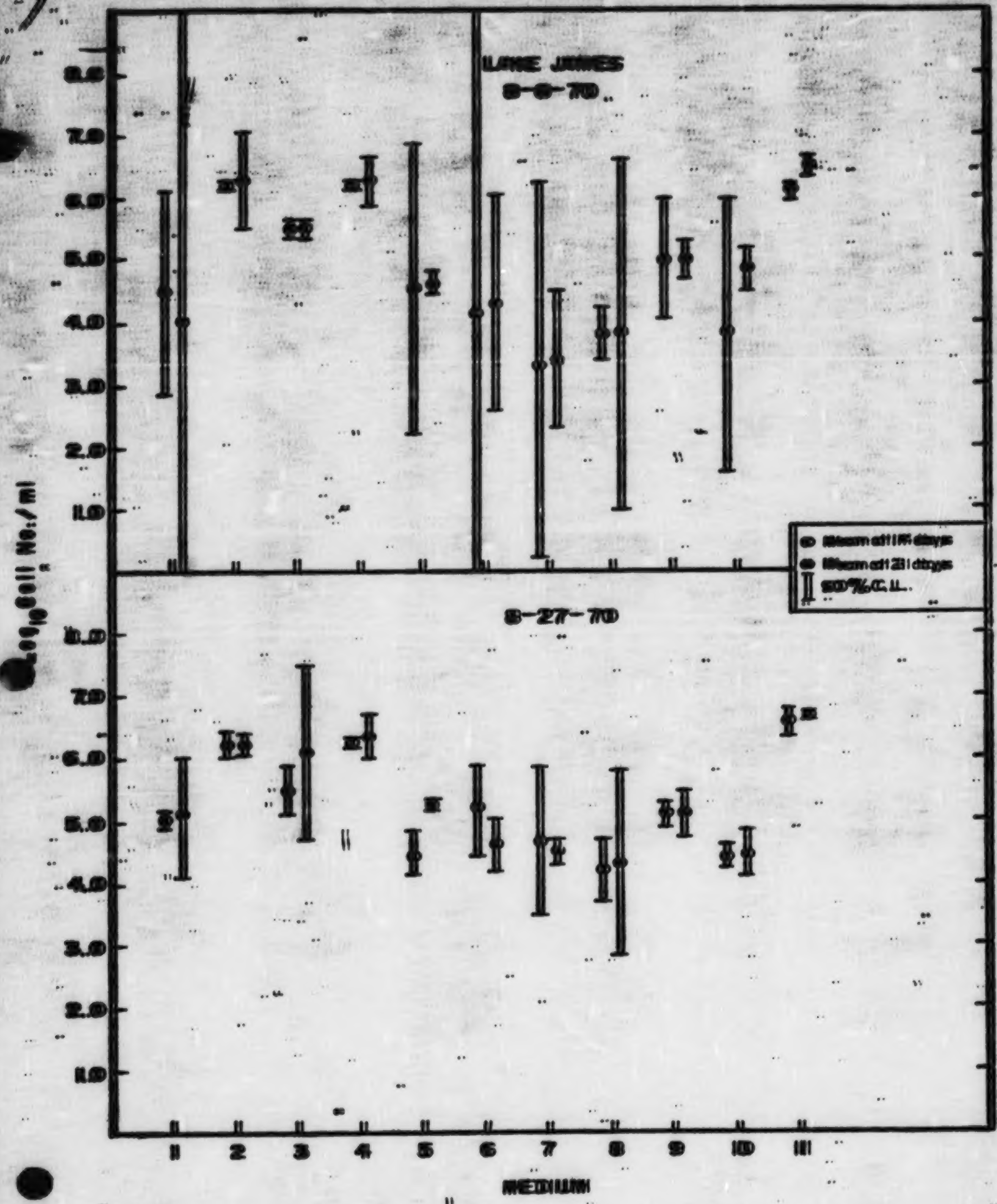

Fig. 21. Log cell number/ml, mean values and 90% confidence limits at 14 and 21 days – Batch Assay, NTA Evaluation.

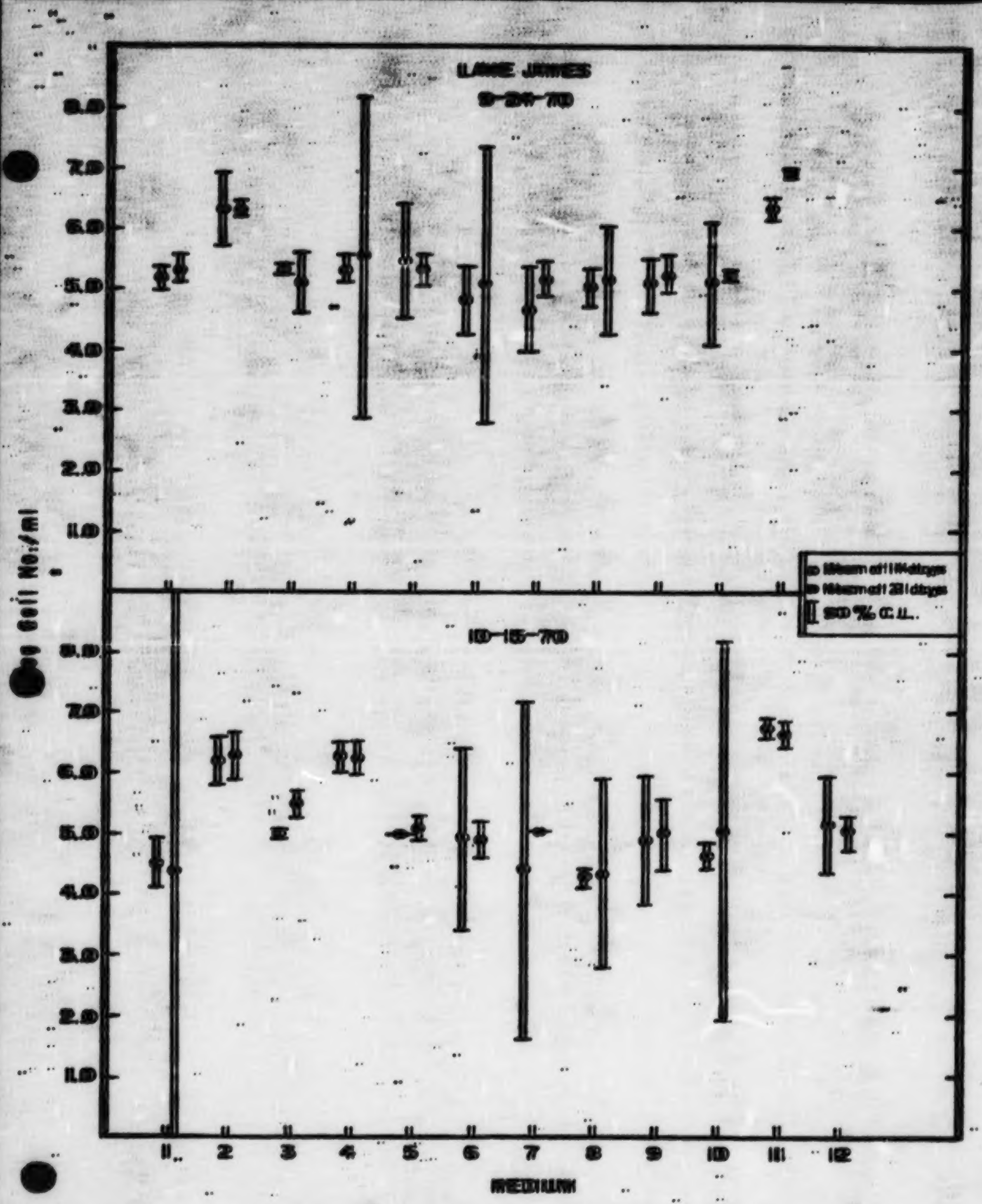

Fig. 22. Log cell number/ml, mean values and 90% confidence limits at 14 and 21 days - Batch Assay, NTA Evaluation.

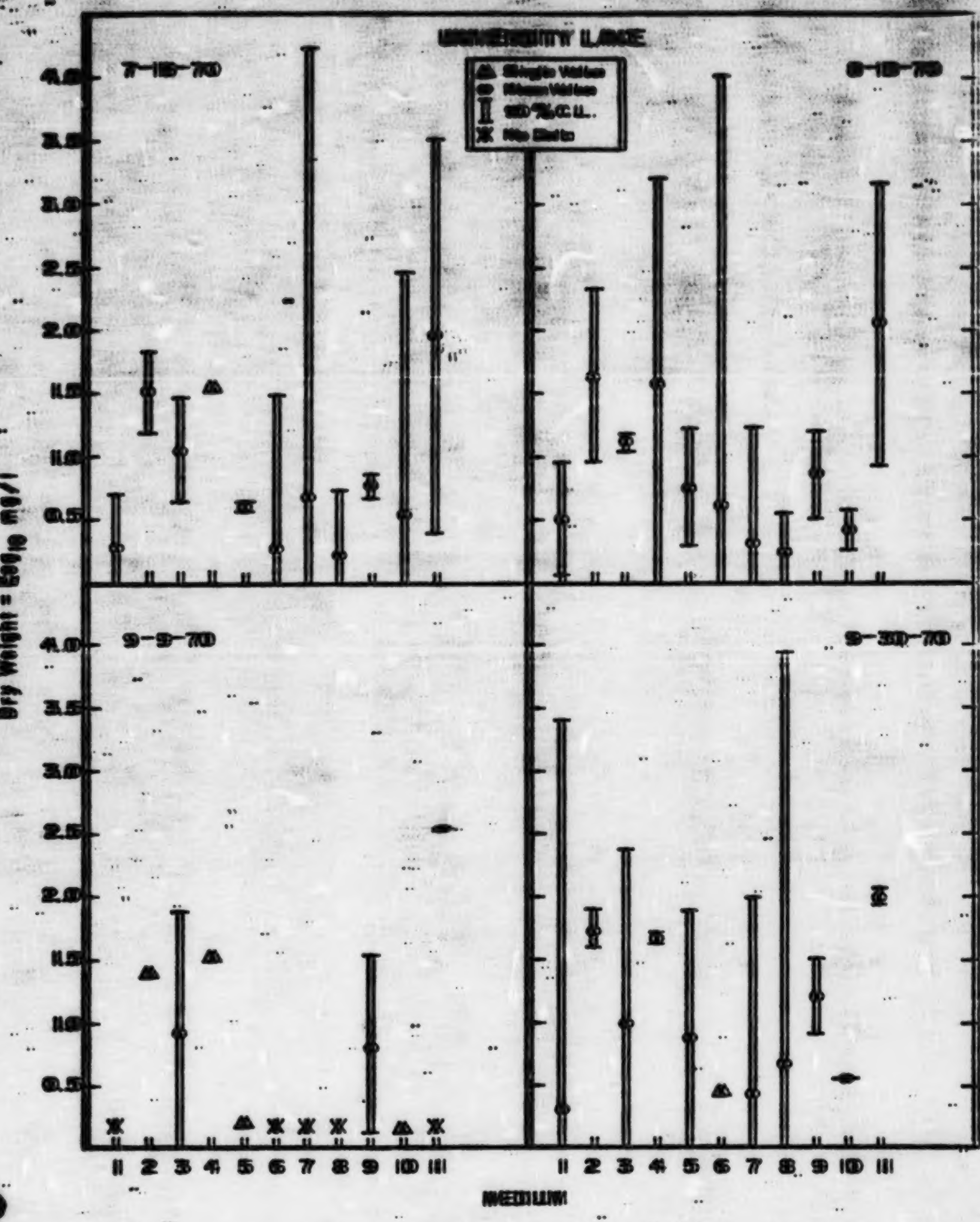

Fig. 23. Log dry weight, mean values and 90% confidence limits at 21 days — Batch Assay, NTA Evaluation.

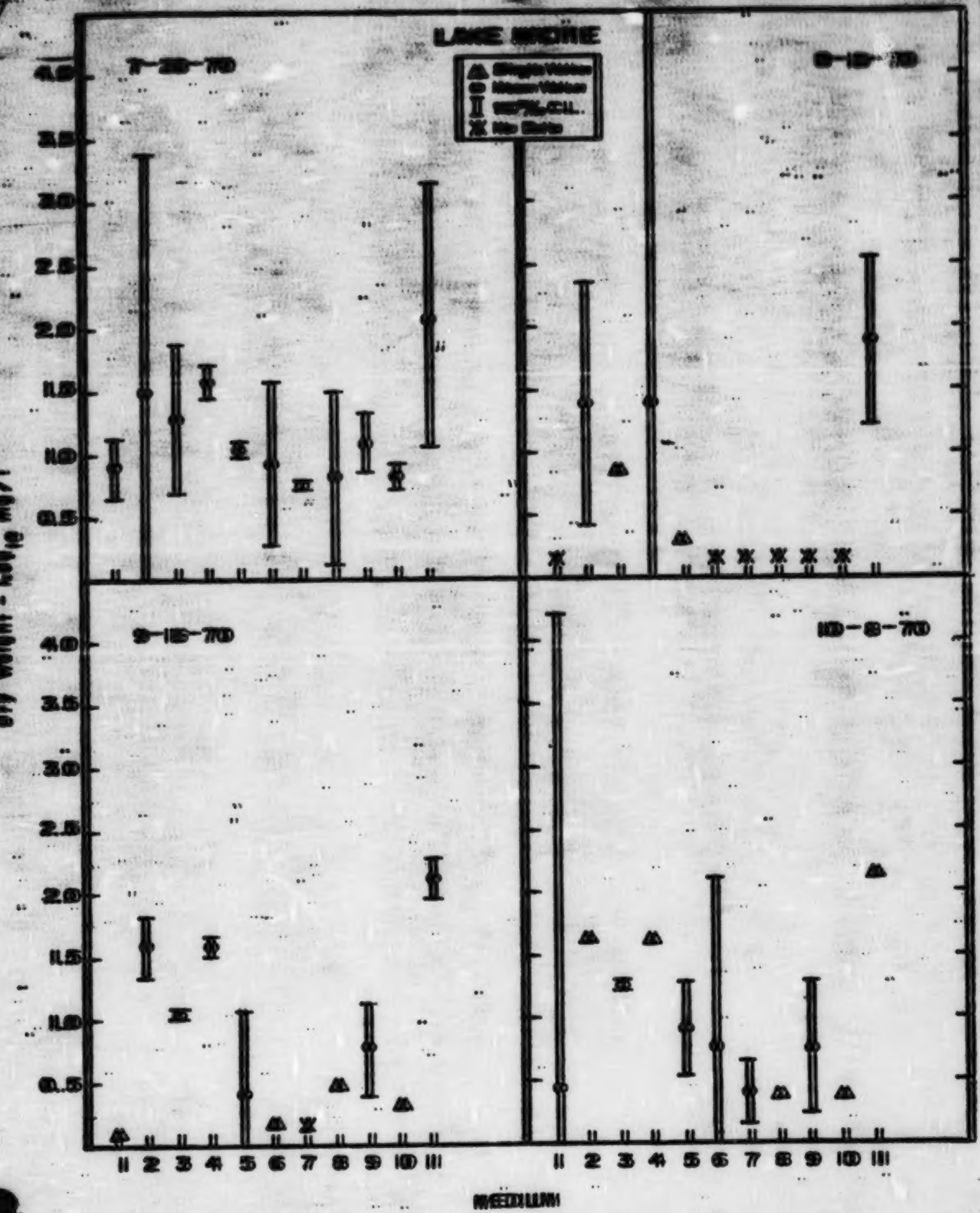

Fig. 24. Log dry weight, mean values and 90% confidence limits at 21 days — Batch Assay, NTA Evaluation.

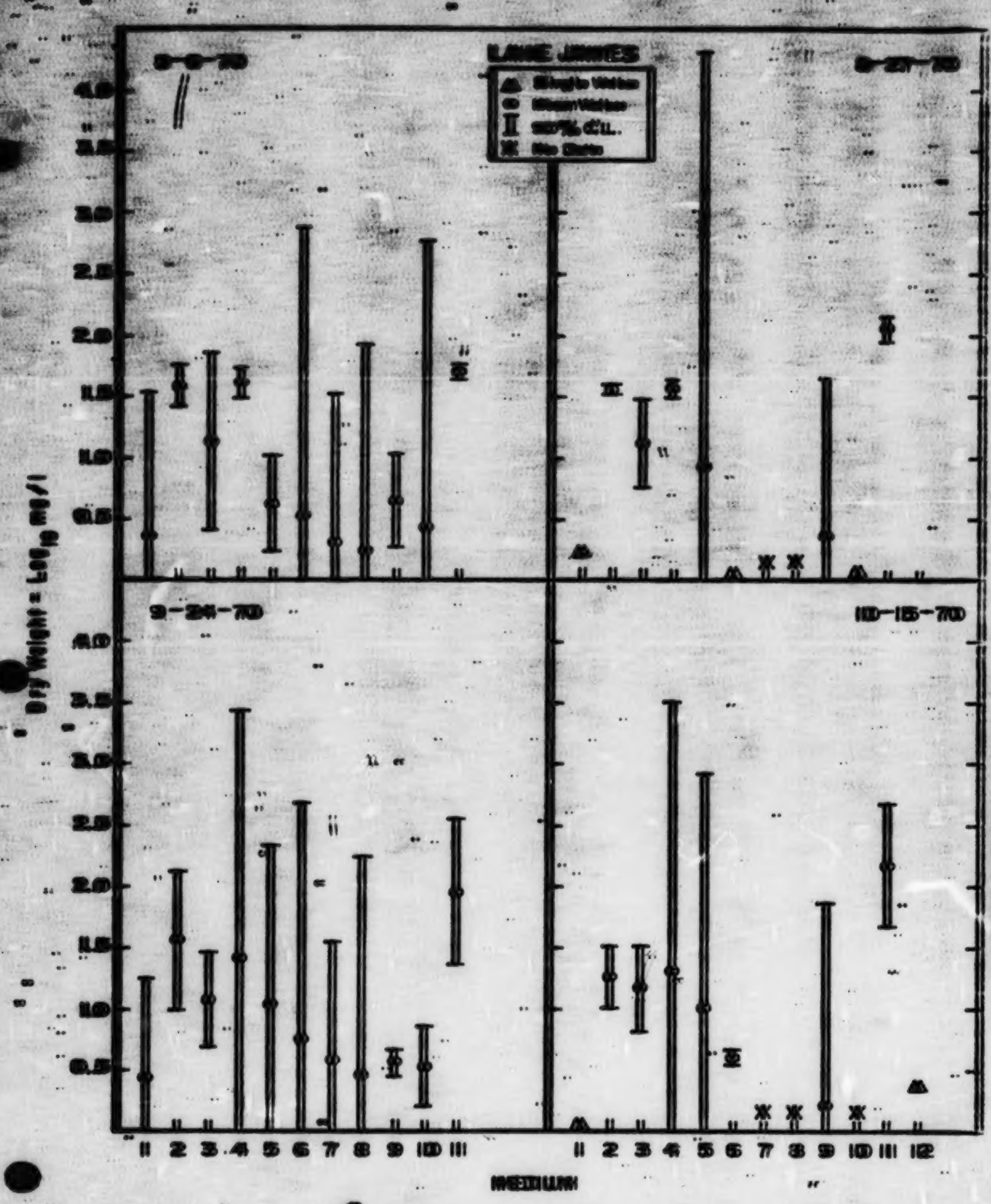

Fig. 25. Log dry weight, mean values and 90% confidence limits at 21 days — Batch Assay, NTA Evaluation.

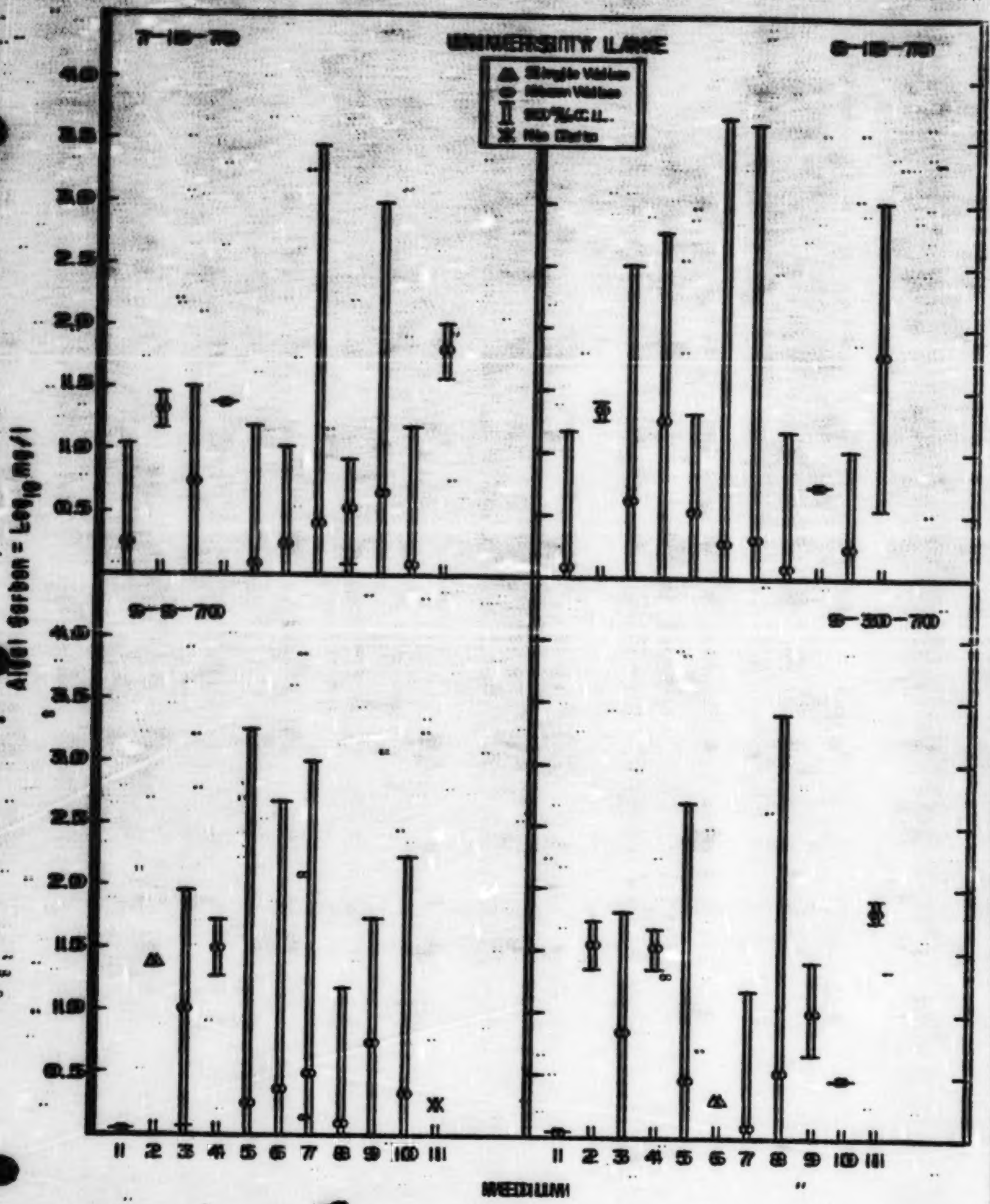

Fig. 21. Log algal carbon, mean values and 90% confidence limits at 21 days — Batch Assay, NTA Evaluation.

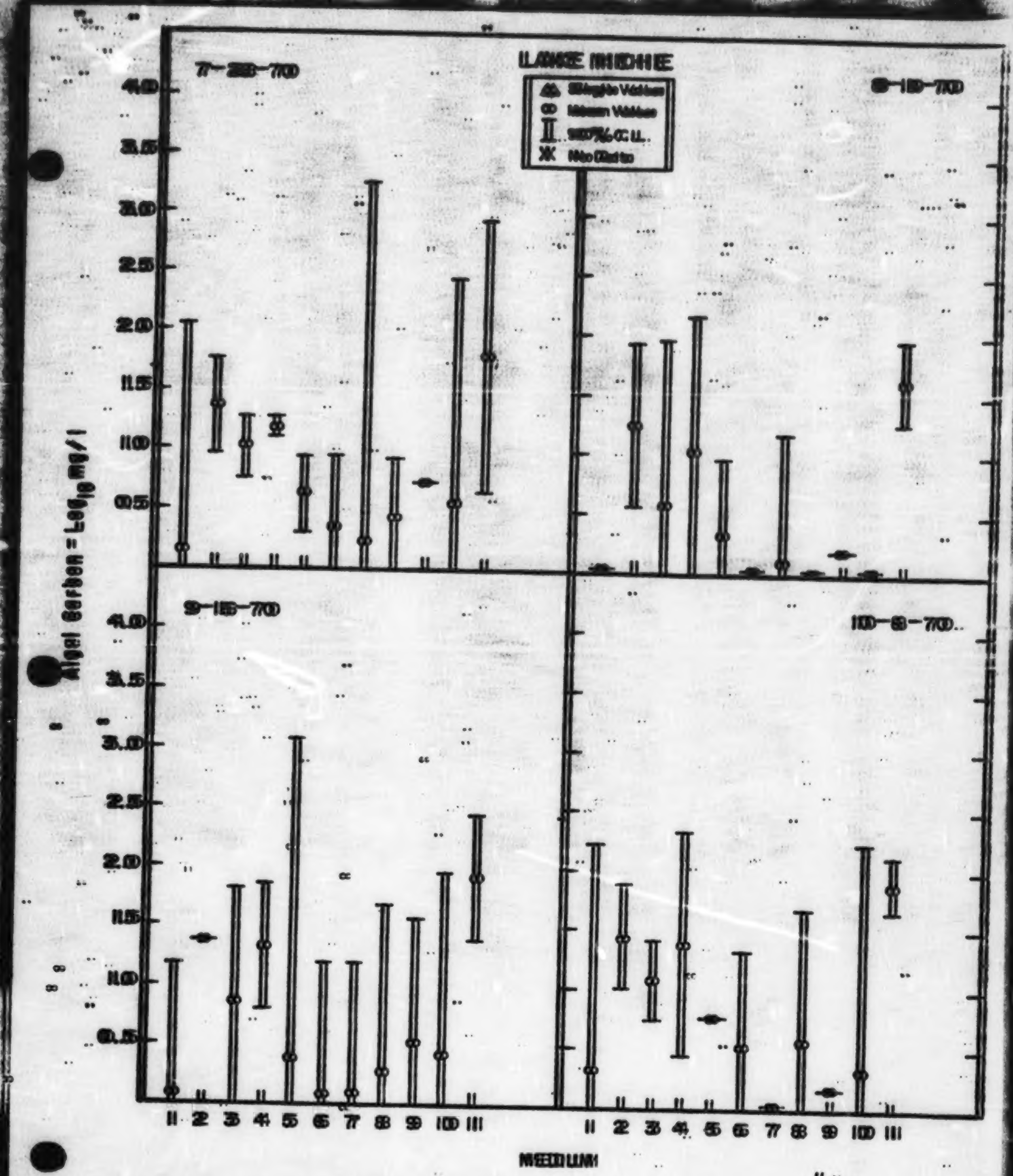

Fig. 27. Log algal carbon, mean values and 90% confidence limits at 21 days — Batch Assay, NTA Evaluation.

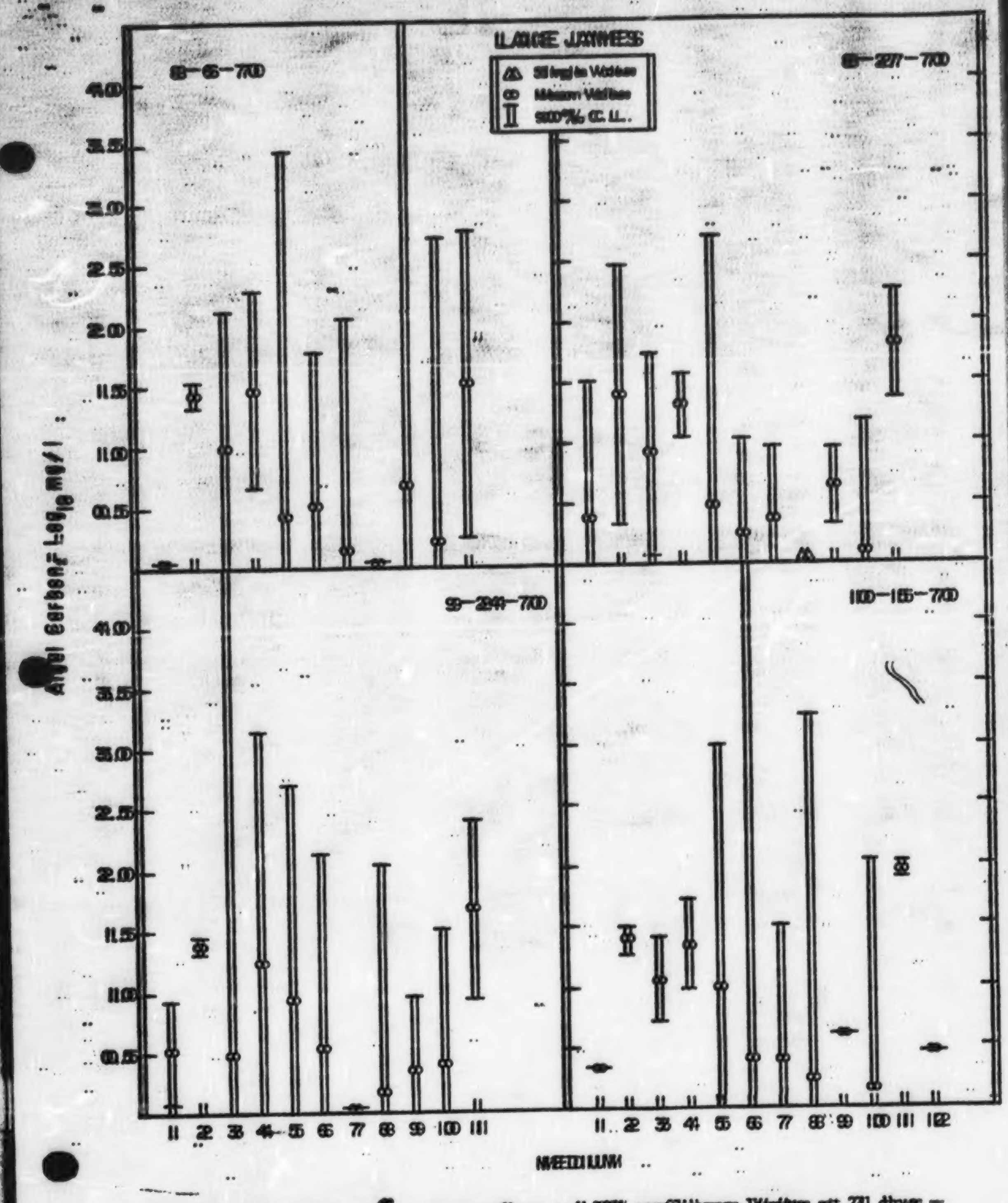

Fig. 28. Log algal carbon, mean values and 90% confidence limits at 21 days — Batch Assay, NTA Evaluation.

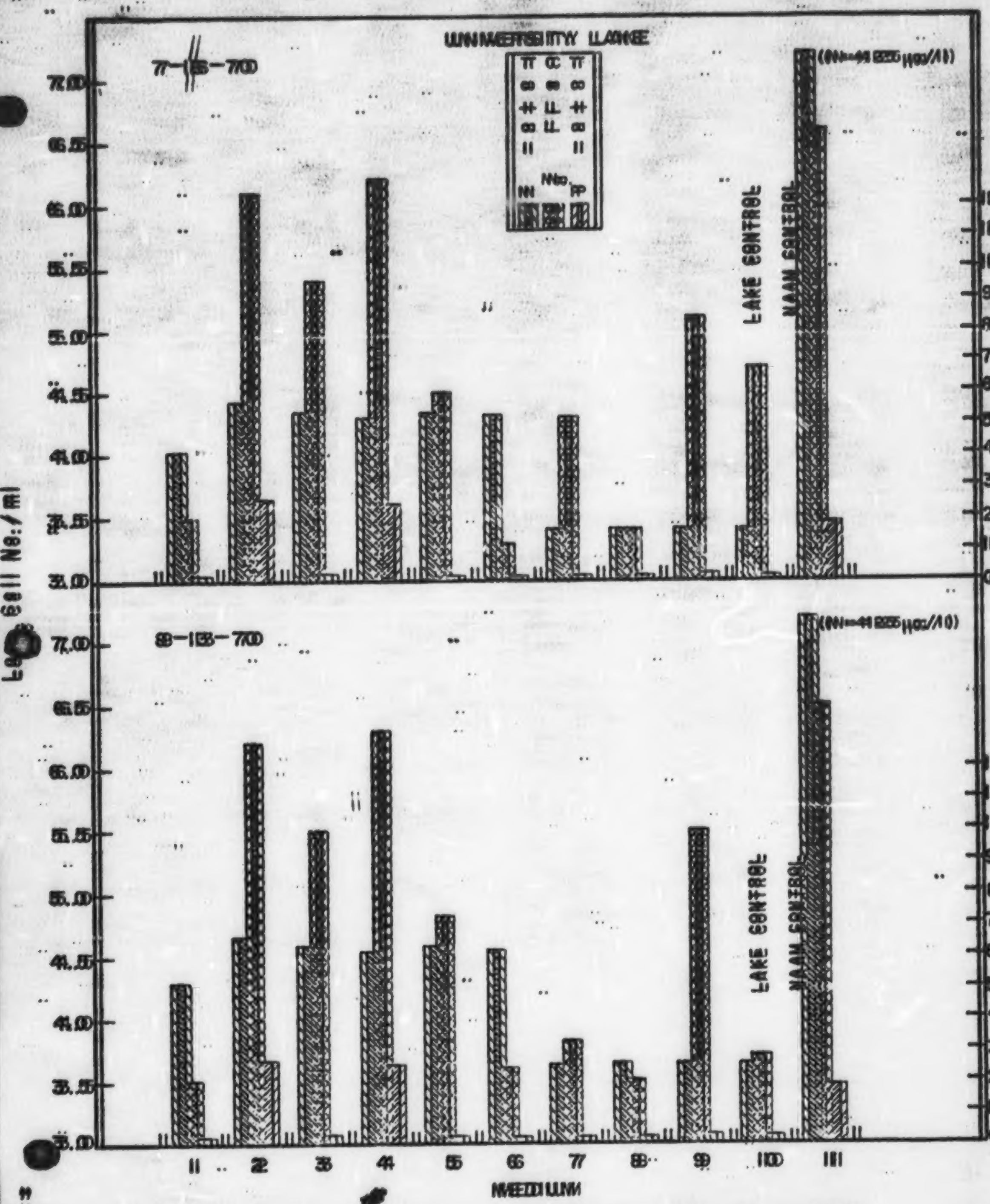

Fig. 29. 21 day growth response of S. capricornutum, log cell number/ml compared to total N and P present in test media – Batch Assay, NTA Evaluation.

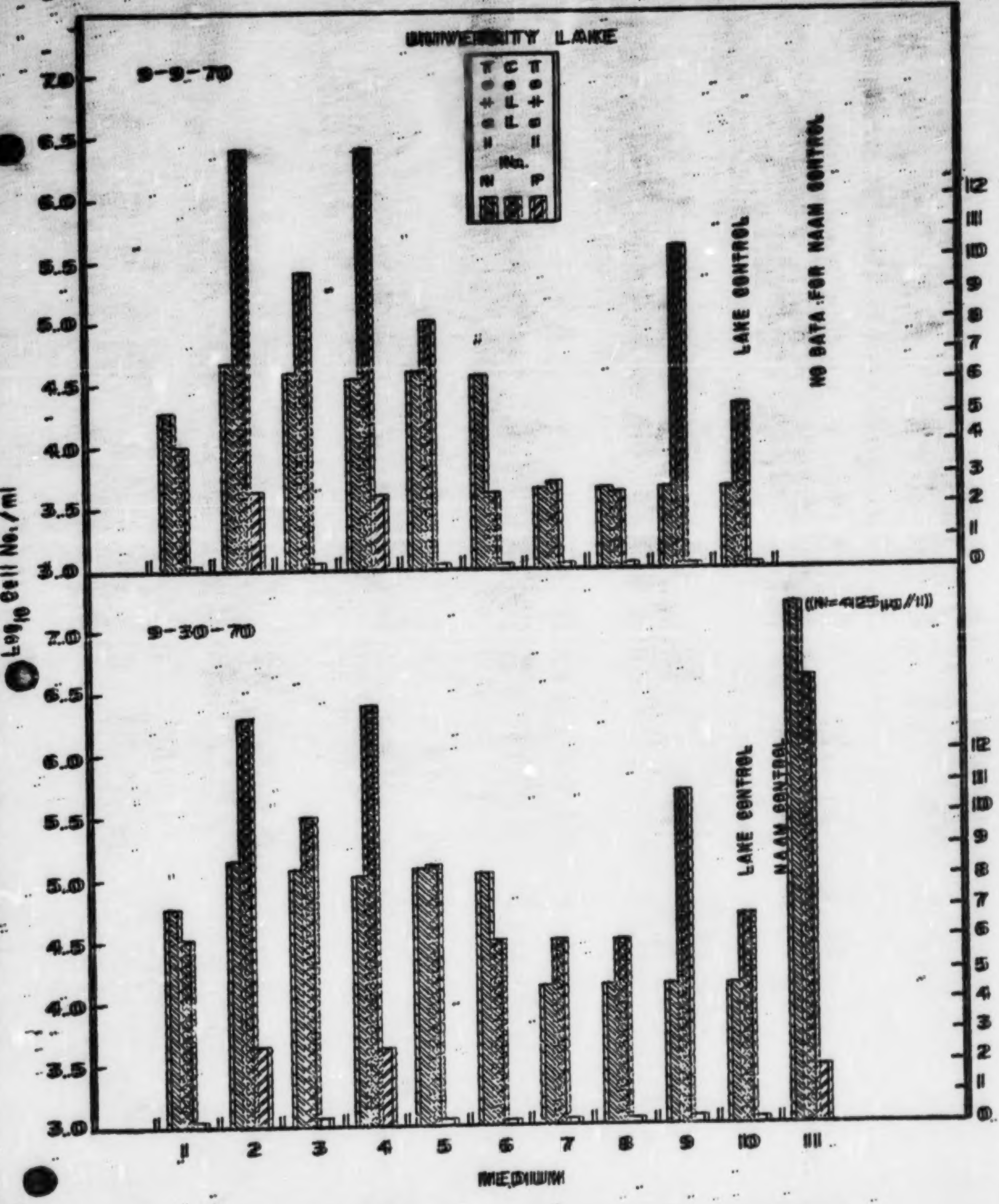

Fig. 30. 21 day growth response of S. capricornutum, log cell number/ml compared to total N and P present in test media - Batch Assay, NTA Evaluation.

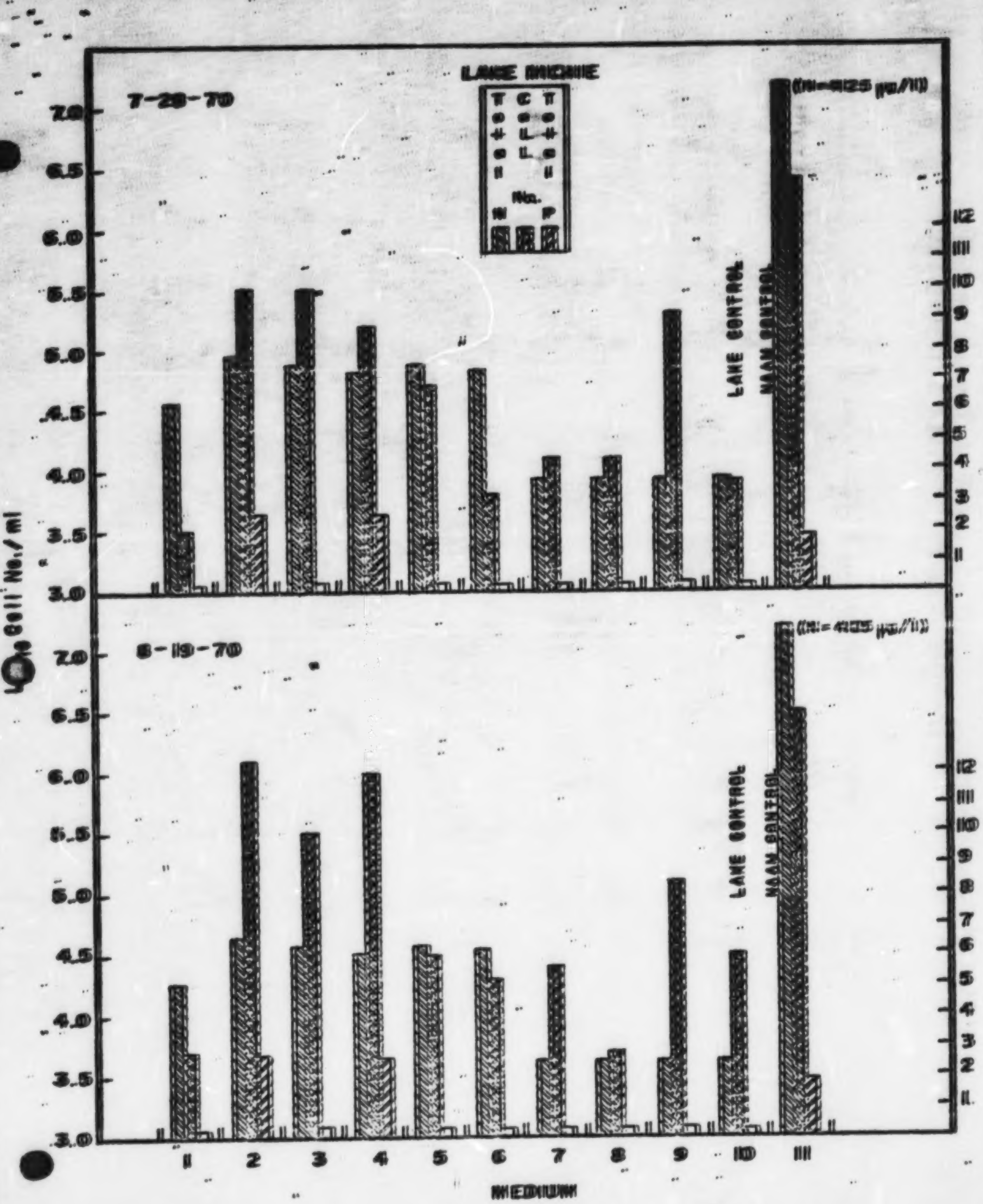

Fig. 31. 21 day growth response of S. capricornutum, log cell number/ml compared to total N and P present in test media – Batch Assay, NTA Evaluation.

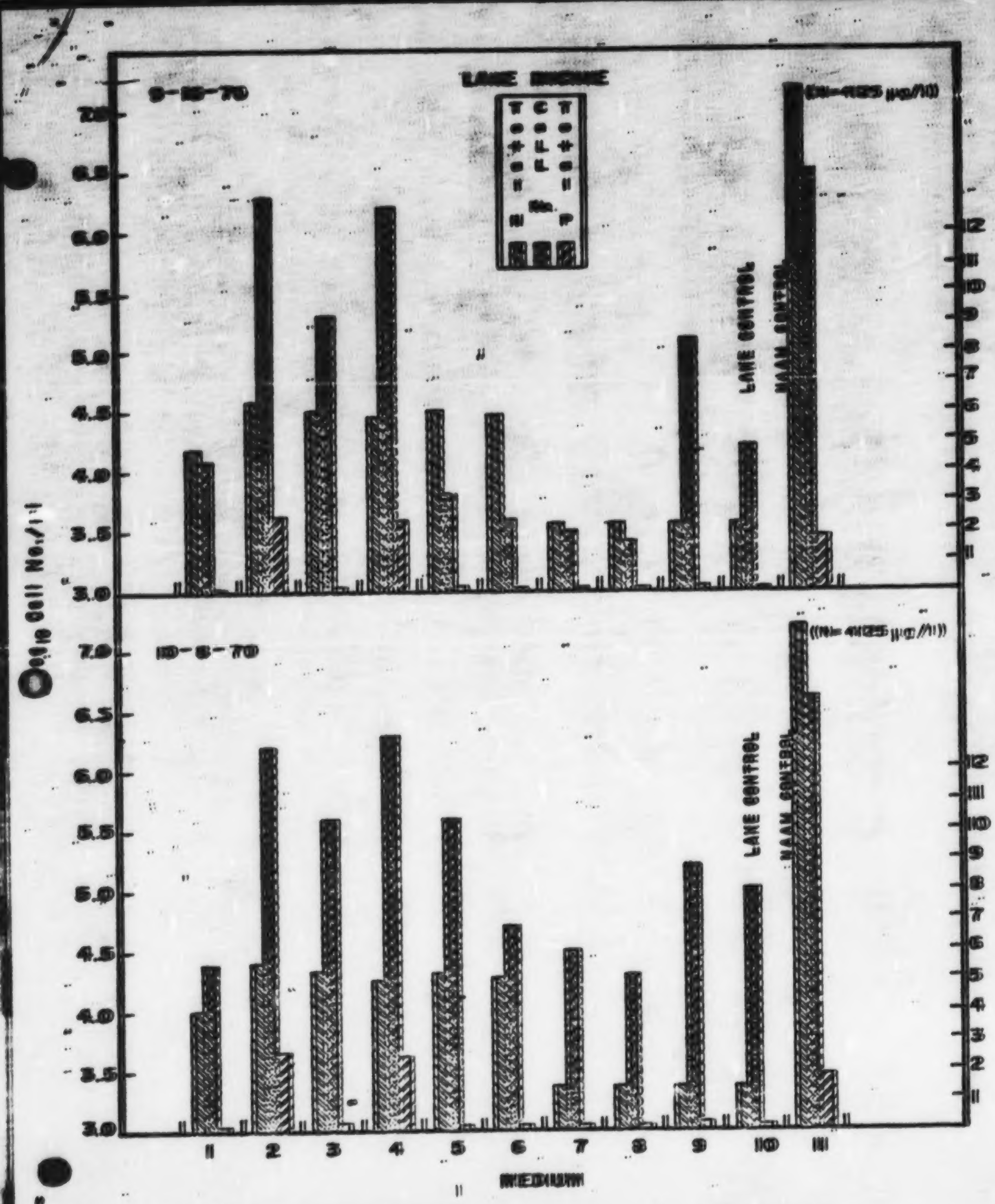

Fig. 32. 21 day growth response of S. capricornutum, log cell number/ml compared to total N and P present in test media – Batch Assay, NTA Evaluation.

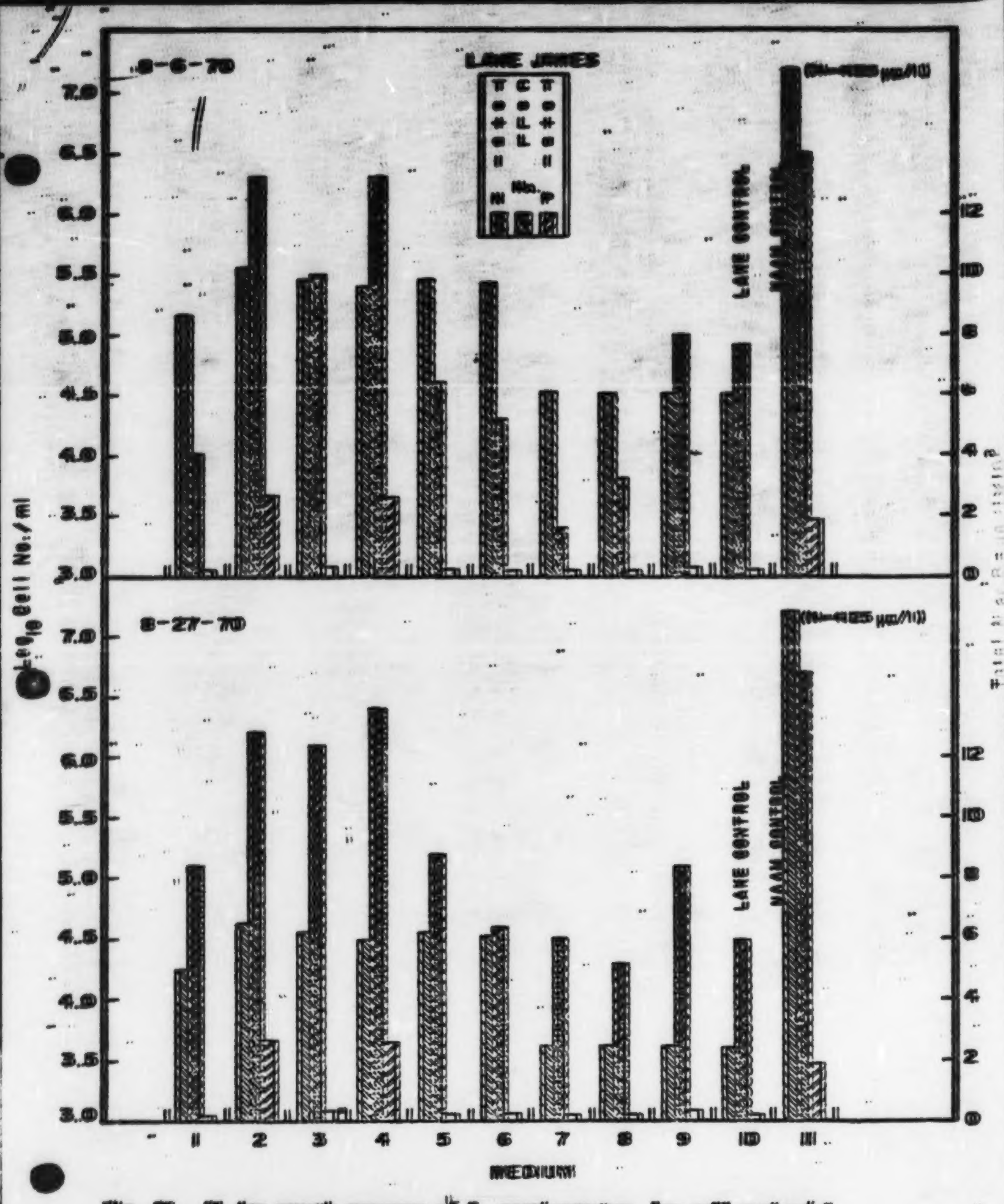

Fig. 33. 21 day growth response of *S. capricornutum*, log cell number/ml compared to total N and P present in test media – Batch Assay. NUA Evaluation.

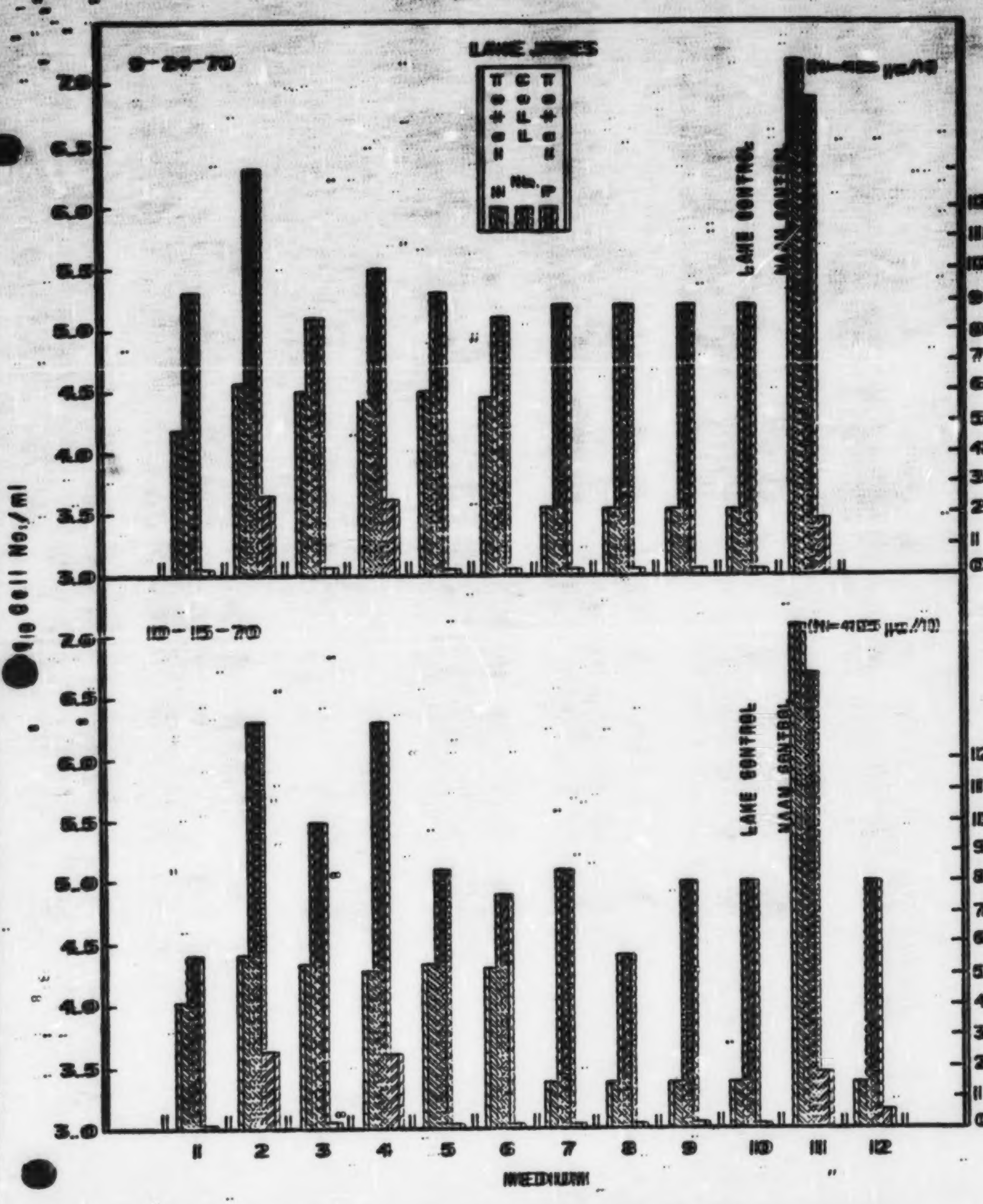

Fig. 34. 21 day growth response of *S. capricornutum*, log cell number/ml compared to total N and P present in test media – Batch Assay, NOA Evaluation.

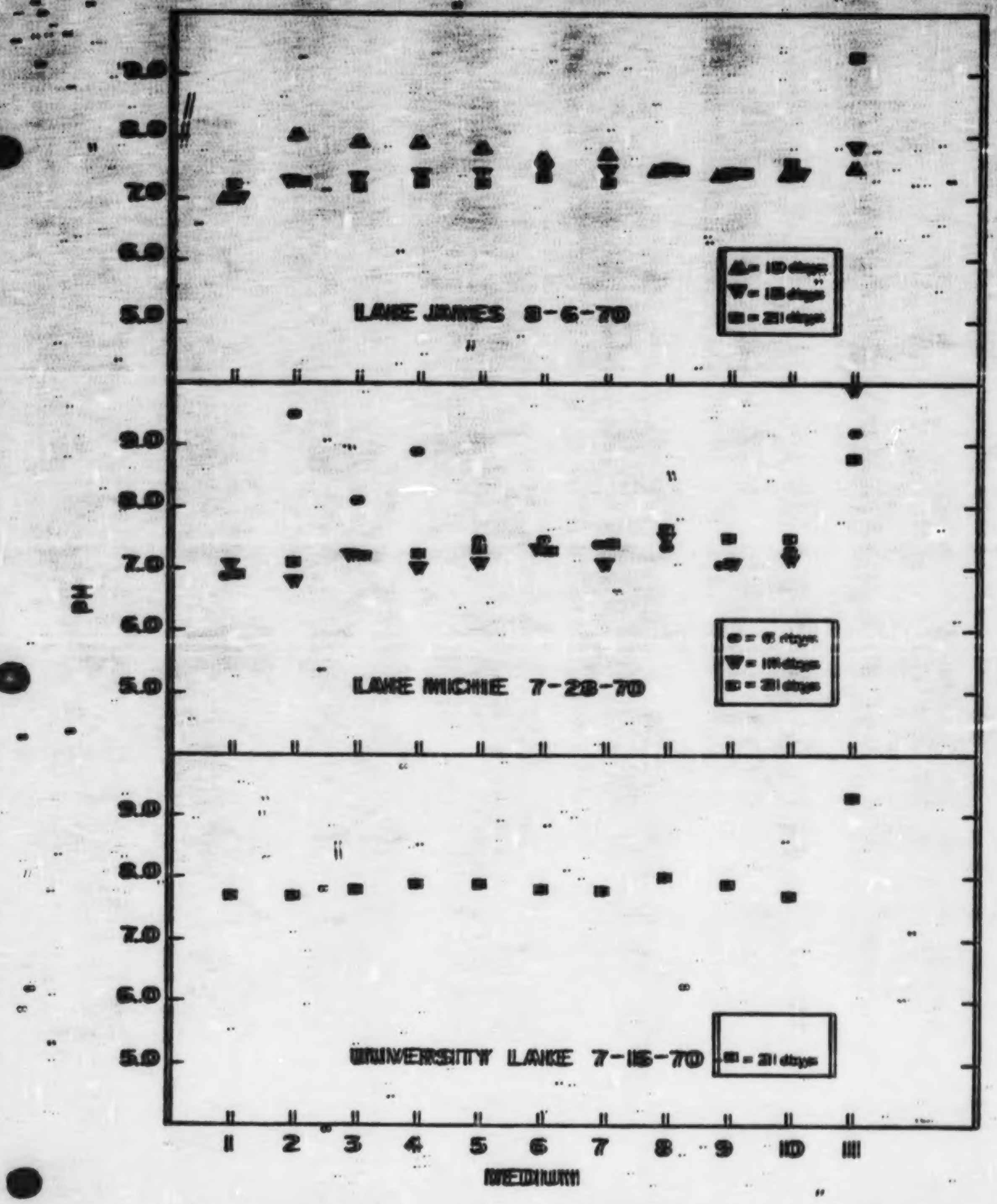

Fig. 15. pH of batch cultures of S. capricornutum at various periods of incubation in test media for NTA Evaluation.

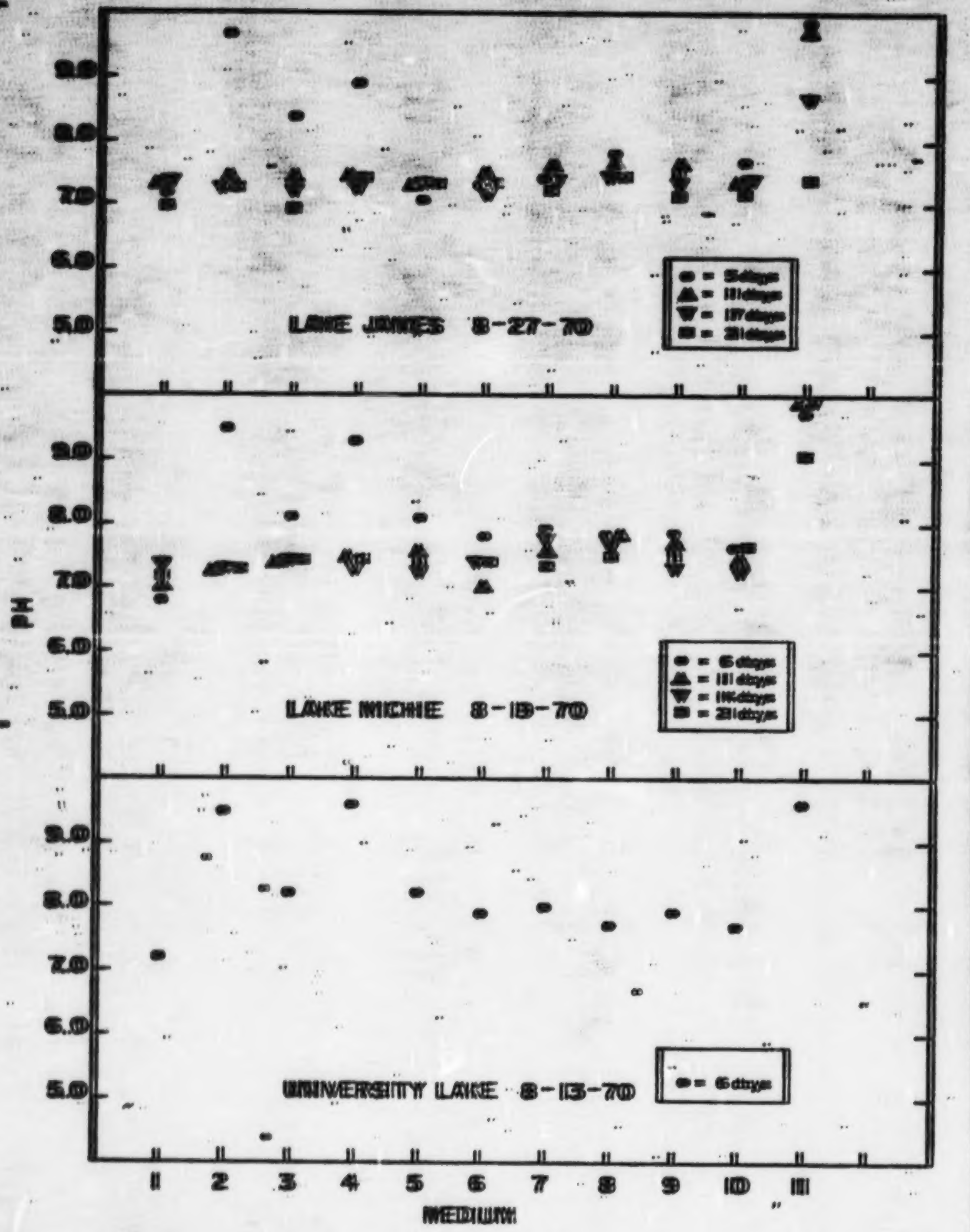

Fig. 35. pH of batch cultures of S. *capricornutum* at various periods of incubation in test media for NTA evaluation.

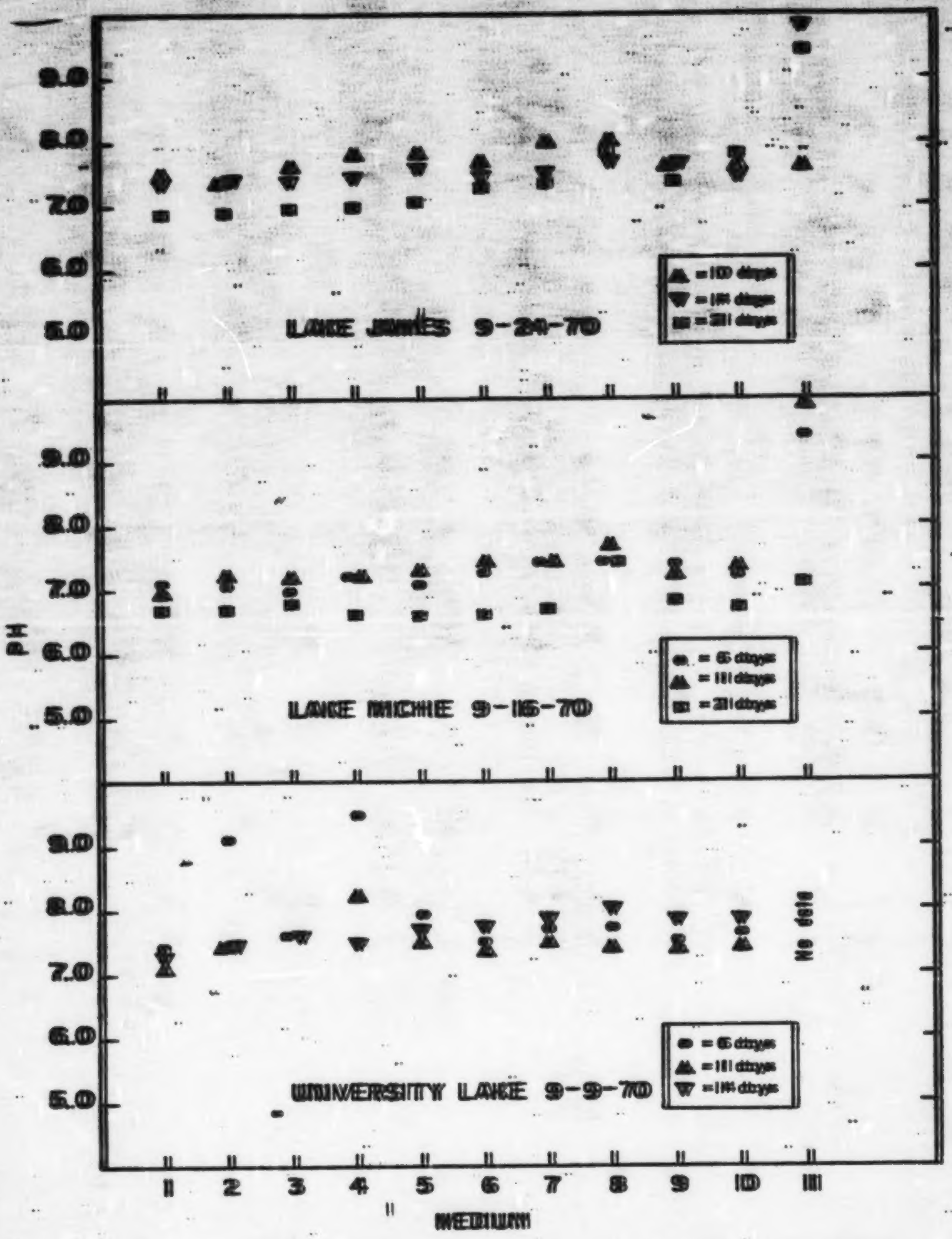

Fig. 37. pH of batch cultures of *S. capricornutum* at various periods of incubation in test media for STM Evaluation.

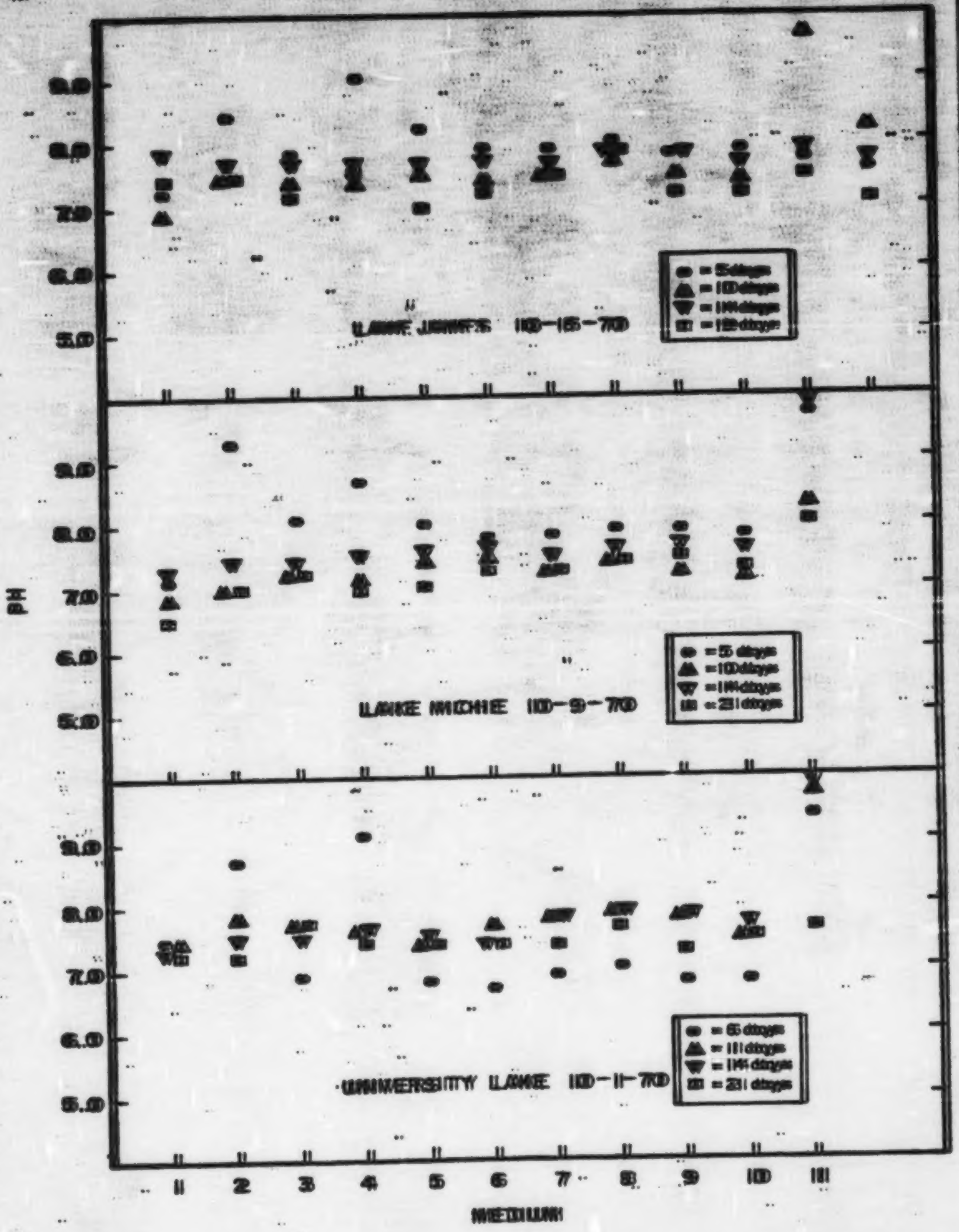

Fig. 38. pH of batch cultures of S. capricornutum at various periods of incubation in test media for NTA Evaluation.

Appendix

NEW ALGAL ASSAY MEDIUM

Macronutrients:

Compound	Conc. (µg/l)	Element	Conc. (µg/l)
$NaNO_3$	25500	N	4200
K_2HPO_4	1044	P	186
$MgCl_2$	5700	Mg	2904
$MgSO_4 \cdot 7\ H_2O$	14700	S	1911
$CaCl_2 \cdot 2\ H_2O$	4410	Ca	1202
$NaHCO_3$	15000	K	469
		Na	11001

Note: If the medium is to be filtered, the trace metal – $FeCl_3$-EDTA mixture is added after filtration. If filtration is not required, K_2HPO_4 should be added last to avoid iron precipitation. Stock solutions of individual salts may be made up in 1000 times the final concentrations.

Micronutrients:

Compound	Conc. (µg/l)	Element	Conc. (µg/l)
H_3BO_3	185.640	B	33.000
$MnCl_2$	264.270	Mn	115.000
$ZnCl_2$	32.700	Zn	15.000
$CoCl_2$	0.780	Co	0.350
$CuCl_2$	0.009	Cu	0.003
$Na_2MoO_4 \cdot 2\ H_2O$	7.260	Mo	2.880
$FeCl_3$	96.000	Fe	33.000
$Na_2EDTA \cdot 2\ H_2O$	300.000		

Note: The trace metals, $FeCl_3$ and EDTA, will be combined in a single stock mix at 1000 times the final concentrations.

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